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13. ABSTRACT (Maximum 200 Words)

Posttraumatic stress disorder (PTSD) is a syndrome of symptoms indicative of emotional dysfunction, which develop after exposure to life-threatening events. Prevalent symptoms are fear and anxiety, which become particularly intense during exposure to situations reminiscent of the traumatic events that precipitated the disease. The amygdala is a key component of the brain's neuronal network that determines the emotional significance of external events. Despite the central role of the amygdala in emotional behavior, little is known about the impact of stress on the amygdala's function. Clinical evidence indicates that norepinephrine and serotonin may participate in the synaptic plasticity phenomena that result in the memory of frightening events in PTSD. Our data indicate that the modulatory effects of norepinephrine and serotonin receptors on synaptic transmission, neuroplasticity and calcium homeostasis are altered in traumatically stressed rat amygdala. The results of this study may aid in the development of new strategies aimed at modifying and preventing the formation of traumatic memory, and thus could be useful for the treatment of combat PTSD in veterans.

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Introduction

Intense or chronic stress can have long-lasting consequences in an individual's health, and can be the cause of debilitating mental illnesses. A very common mental illness induced by traumatic stress is posttraumatic stress disorder (PTSD). PTSD is a syndrome of symptoms indicative of emotional dysfunction, which develop after exposure to life-threatening events, or very stressful situations of different nature. Prevalent symptoms are fear and anxiety, which become particularly intense during exposure to situations reminiscent of the traumatic events that precipitated the disease. PTSD is a psychiatric disorder of considerable prevalence and morbidity and can affect persons of any age and ethnic or socioeconomic background. It is, unfortunately, a far too common result of participation in wars. In addition, epidemiological studies have suggested that the prevalence of PTSD is even higher in inner city communities exposed to compound community trauma. It is imperative, therefore, to understand the neurobiological mechanisms by which exposure to traumatic stress leads to PTSD in order to foster the development of new therapeutic strategies for the prevention and treatment of stress-related affective disorders such as PTSD.

Clinical evidence indicates that certain stress-related affective disorders such as PTSD are associated with changes in the amygdala's excitability. The amygdala is a key component of the brain's neuronal network that determines the emotional significance of external events (LeDoux, 1992; Davis, 1994; Breiter et al., 1996; Schneider et al., 1997; LaBar et al., 1998; Buchel et al., 1998; Whalen et al., 1998; Baird et al., 1999; Davidson et al., 1999; Davidson and Slagter, 2000; Buchel and Dolan, 2000). Via efferent pathways to the hypothalamus, the amygdala can trigger the neuroendocrine cascades that are part of the stress response (Habib et al., 2001; Pitkänen, 2000; Davis, 1992; Davis et al., 1994) and via reciprocal connections with the cerebral cortex and limbic structures, it modulates the orchestration of the behavioral response (Goldstein et al., 1996; Pitkänen, 2000). Despite the central role of the amygdala in emotional behavior, little is known about the impact of stress on amygdala function.

The amygdala is a brain region critical brain critical to the consolidation of emotional memory; thus, in fear conditioning, an experimental paradigm that shares characteristics of anxiety disorders and PTSD, the amygdala mediates the learning experience and is likely to be the site of memory storage. Evidence supporting this view is as follows: 1) Fear-conditioning is associated with a strong activation of the amygdala in both rats and humans, 2) Parallel to the development of the conditioned fear-behavior, there is a long-lasting potentiation of evoked field potentials or synaptic currents of amygdala neurons. 3) Amygdala lesions severely impair or block both the acquisition and expression of conditioned fear. In other types of unconditioned emotional experiences, the amygdala is believed to play a central role in modulating the consolidation of emotional memories in the cerebral cortex, as well as in modulating the function of other limbic structures involved in memory formation (Kim et al., 2001).

Increased release of norepinephrine and serotonin in the amygdala is associated with behaviors that are typically seen during states of fear, suggesting that norepinephrine and serotonin may play a role in amygdala circuits mediating fear responses (Servatius et al., 1995). It has been speculated that norepinephrine and serotonin may also participate in the synaptic plasticity phenomena that result in the memory of frightening events and also in PTSD. The short-term effects and long-term consequences of stress-induced excessive norepinephrine and serotonin release on amygdala physiology are unknown.

The studies proposed in this grant focus on the roles of norepinephrine and serotonin receptors on activity-dependent neuroplasticity and calcium signaling in amygdala slice preparations from traumatically stressed and control rats. The hypothesis we have raised is that the modulatory effects of norepinephrine and serotonin receptors on synaptic transmission, neuroplasticity and calcium homeostasis are altered in traumatically stressed rat amygdala. The results of this study may aid in the development of new strategies aimed at modifying and preventing the formation of traumatic memory, and thus could be useful for the treatment of combat PTSD in veterans.

Body

In the fourth year of this project we have concentrated our efforts in identifying the neurobiological alterations in amygdala physiology and function induced by traumatic stress. Specifically, we have discovered one of the mechanisms underlying the stress-induced hyperexcitability in the amygdala. We have found that stress impairs the function of a specific subtype of adrenergic receptors (the α_{1A} adrenoceptor) that mediates the actions of noradrenaline (a neurotransmitter that plays a central role in the stress response). Activation of these receptors by noradrenaline facilitates inhibitory activity in the amygdala, preventing over-excitation and suppressing synaptic neuronal plasticity that takes place during memory formation. The stress-induced impairment in the function of these receptors will therefore result in neuronal hyperexcitability and hyper-responsiveness in the amygdala, and will facilitate the "registration" of memories associated with emotionally significant events.

These findings provide, for the first time, direct evidence that stress causes impairment in the modulation of inhibitory activity in the brain, and thus offer an important insight into the possible mechanisms underlying the hyperexcitability and hyperesponsiveness of the amygdala in certain stress-related mental illnesses such as PTSD, as well as in the stress-induced exacerbation of seizure activity in epileptic patients. These important findings have been recentely published in ***Neuropsychopharmacology* 29 (1): 45-58, 2004**. In addition, the abstract of this work was presented at the ***Faculty Senate Research Day and Graduate Student Colloquia* (From Bench to Bedside and Battlefield: Translational Research at the Nation's Medical School)** at USUHS, Bethesda, MD. This poster was selected from more than 150 abstracts submitted by researchers from the Uniformed Services University and its affiliates to be presented at the ***PRESIDENT'S POSTER SESSION.*** The selection was based on the high-impact military and civilian biomedical research conducted at USUHS and its affiliates.

The same abstract was submitted for presentation at the Society for Neuroscience meeting in New Orleans (Novemeber, 2003). Again, our abstract was one of 600 (among more than 15,000 submissions from all over the world) requested by the **Public Information Committee** for inclusion in the **Annual Meeting Press Book** as a lay-language summary. These summaries are used to set up press interviews with scientists whose work is found to have a major impact on a specific research field.

Because of the important implications of our findings to the neurobiological basis of PTSD, we have requested and been granted a one-year extension of this project in order to **elucidate the mechanisms involved in the stress-induced impairment of α_{1A} adrenoceptor function.** To accomplish this we will use immunofluorescence, western blotting and receptor binding techniques, as well as patch-clamp techniques, to determine

whether α_{1A} adrenoceptors undergo downregulation, internalization or desensitization after stress exposure, or if alterations occur in the intracellular signaling pathways that are activated by these receptors.

During this forth year we have also investigated the effects of traumatic stress on the function of α_2 and β adrenoceptor subtypes. By using field potential recordings, we have been able to determine the relative consequence of each adrenoceptor subtype activation on the effects of norepinephrene in the overall excitability of the amygdala in control and stressed rats.

Detailed description of research accomplishments

An animal model for studying stress-related disorders

Traumatic stress can have long-lasting consequences for an individual and can result in affective disorders such as PTSD. No preventive treatment currently exists for PTSD. The development of an animal model is critical for the study of therapeutic and prophylactic treatments. Historically, effective therapeutic treatments have been developed for diseases only if adequate animal models were available. We have successfully established and tested the inescapable tail-shock model of stress in rats and verified that short and long-lasting behavioral and physiological alterations, known to be mediated by the amygdala, resulted from applying the inescapable tail-shock stress paradigm to the animals studied. The behavioral and neurobiological alterations induced by this rat model are similar to those found in PTSD patients (Table 1 below).

In our model, stress exposure consists of a two-hour per day session of immobilization and tail-shocks, for three consecutives days. The animals are restrained in a plexiglas tube, and 40 electric shocks (2 mA, 3 s duration) are applied at varying intervals (140 to 180 s). This stress protocol was adapted from the "learned helplessness" paradigm in which animals undergo an aversive experience under conditions in which they cannot perform any adaptive response (Seligman and Maier, 1967; Maier and Seligman, 1976). We stress the rats for three consecutive days because it has been previously demonstrated that repeated immobilization and tail-shock stress sessions for three days is more effective that a single stress session in producing physiological and behavioral abnormalities, such as elevations in basal plasma corticosterone levels, exaggerated acoustic startle responses and reduced body weight. Further exposure to stress does not appear to result in greater physiological and behavioral changes.

In our study, body weight of the stressed group was 45 ± 2.1 g ($n = 21$) before the first stress session, and 54.5 ± 2.8 g ($n = 18$) after the last stress session (Figure 1). Control rats, weighted at the same time of the day before the first stress session, were 46 ± 1.7 g ($n = 24$), and 63 ± 2.1 g ($n = 21$) after the last stress session (Figure 1). The difference in body weight between stressed and control rats after the last stress session was statistically significant ($p< 0.05$) and stressed rats continued to display reduced body weight gain for as long as body weight was monitored (up to 10 days after stressor cessation).

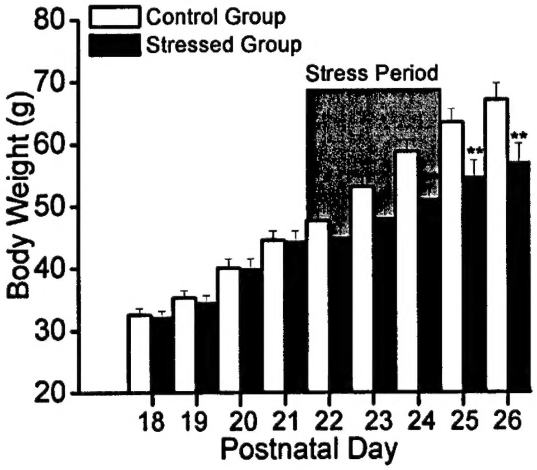


Figure 1. Restrain/tail-shock stress reduces body weight gain. Exposure to stress on postnatal days (PDN) 22, 23 and 24 reduced body weigh gain. Body weight difference between control and stressed rats was statistically significant after the first day of stress (**p< 0.01). Data on PDN 26 are from rats that were not used for electrophysiological experiments. Sample sizes range from 12 (PDN 26) to 24 rats.

We have also assessed the effectiveness of our tail-shock model of stress in rats by measuring behavioral response to acoustic startle, and circulating corticosterone levels. The group of rats receiving restraint and tail shock had potentiated startle responses from the 4th day through the 10th day following stress exposure (26.7 to 66.7% greater than the group receiving no treatment, n = 26, $p<0.05$). Tail blood samples were collected and assayed for corticosterone levels at three time points during the study: a) immediately before stress, b) after the first and c) after the third day of stress. All stressed groups displayed elevated basal corticosterone levels (41 ±4.2% and 53 ±43.8%, n = 24, $p<0.05$) confirming previously published studies published by Servatius et al., 1995. Thus, our results of the inescapable tail shock paradigm as a model of PTSD are consistent with those in the literature (Servatius et al, 1995) and indicate that we have successfully established and tested the inescapable tail-shock model of stress in rats.

Table 1. Comparison of Symptoms of PTSD in Humans to Dysfunction Related to Stress in Rats

PTSD in Humans	Inescapable tail-shock model of stress in rats
Weight loss	Suppressed feeding and body weight loss
Difficulty falling or staying asleep, nightmares	Altered sleep patterns
Psychomotor numbness	Persistent behavioral abnormalities i.e. suppressed open-field activity, longer hanging wire latencies
Poor concentration; memory deficits	Deficits in escape/avoidance learning and learning of an appetitive task
Hypervigilance and/or exaggerated startle reponse	Exaggerated startle
Hyperresponsiveness of the noradrenergic system	Hyperresponsiveness of the noradrenergic system

Stress impairs α_{1A} adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala.

Intense or chronic stress can produce pathophysiological alterations in the systems involved in the stress response. The amygdala is a key component of the brain's neuronal network that processes and assigns emotional value to life's experiences, consolidates the memory of emotionally significant events, and organizes the behavioral response to these events. Clinical evidence indicates that certain stress-related affective disorders are associated with changes in amygdala excitability, implicating possible dysfunction of the GABAergic system. An integral component of the stress response is the activation of the noradrenergic system. Norepinephrine (NE), acting via α_1 adrenoceptors, modulates GABAergic inhibition. Despite evidence for stress-related impairments in both α_1 adrenoceptor function and GABAergic transmission, as well evidence that α_1 adrenoceptors mediate the noradrenergic modulation of GABAergic transmission, an association between stress, impaired function of α_1 adrenoceptors, and pathophysiological alterations in GABAergic inhibition has not been previously considered.

Recently we investigated whether NE modulates GABAergic transmission in the basolateral nucleus of the amygdala (BLA), and if so, whether noradrenergic modulation of GABAergic transmission was altered by exposure to traumatic stress. We studied the BLA because this amygdala region is heavily involved in the processing of emotional experiences, as it receives both direct and indirect thalamic and cortical inputs and it is extensively interconnected with the prefrontal/frontal cortex and the hippocampus (Pitkänen, 2000). Furthermore, it appears that the BLA selectively (among the different amygdala nuclei) modulates the consolidation of emotional memories. Our results show that NE facilitates spontaneous, evoked, and action potential-independent, quantal GABA release in the BLA via α_{1A} adrenoceptors, and that these effects of NE are virtually absent in stressed rats.

Stress blocks noradrenergic facilitation of GABAergic synaptic transmission.

Noradrenergic modulation of spontaneous IPSCs

To investigate whether NE modulates GABAergic transmission in the BLA, and whether stress alters this modulation, we first examined the effects of NE on action-potential dependent, spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from BLA pyramidal neurons, in control and stressed rats. Spontaneous IPSCs were recorded at a holding potential of –70 mV, and in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and yohimbine (20 μM) to block NMDA, AMPA/kainate, β and α_2 receptors, respectively.

In control rats, the mean frequency of sIPSCs recorded in the soma of BLA pyramidal neurons was 3.1 ± 1.6 Hz (n = 21). Bath application of bicuculline (10 μM) eliminated sIPSCs, confirming that they were mediated by $GABA_A$ receptors. NE (10 μM) caused a significant increase of the mean sIPSC frequency (984.3.9 ± 148.2% of the control values, n = 21, $p<0.01$; Fig. 2A) and amplitude (144.0 ± 12.8% of the control values, n = 21, $p<0.05$; Fig. 2A) that persisted throughout the application of NE and was completely reversed after removal of the agonist. These effects of NE were not accompanied by any significant change in the rise time or decay time constant of sIPSCs (Fig. 2A), and were blocked by the α_1 adrenoceptor antagonist prazosin (1 μM, Fig. 2C) confirming that NE was acting via α_1 adrenergic receptors. In stressed rats, the mean frequency of spontaneous IPSCs was 2.6 ± 2.3 Hz. NE (10 μM) had no significant effect on the frequency or amplitude of sIPSCs. Thus, in the presence of NE (10 μM), the frequency of sIPSCs was 128.9 ± 19.2% and the amplitude was 111.4 ± 10.2% of control values (n = 19, Fig. 2B). In addition, bath perfusion of NE (10 μM) caused no significant changes in the kinetics of these currents (rise time and decay time constant of sIPSCs; Fig. 2B).

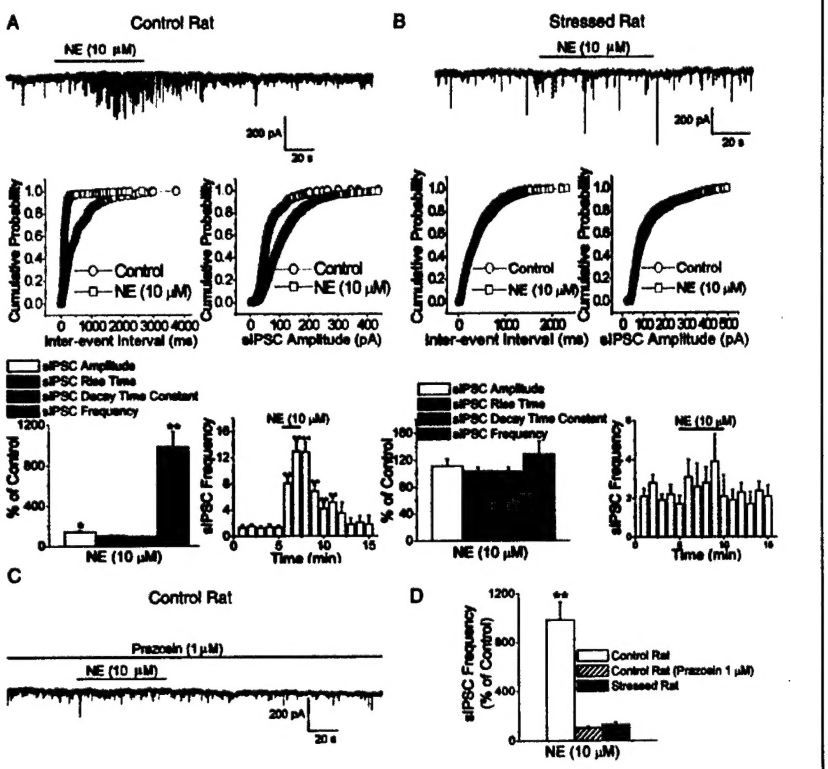


Figure 2. Activation of α_1 adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (A) Top trace: Effects of NE on spontaneous IPSCs recorded from a BLA pyramidal cell of a control rat. (B) Top trace: sIPSCs recorded from a BLA pyramidal cell of a stressed rat. Middle graphs: Cumulative probability plots of inter-event intervals and amplitude of sIPSC. Bottom graphs: (C) Prazosin prevented the NE-induced increase of sIPSCs. (D) Bar graph shows the effects of NE on the mean frequency of sIPSCs recorded from control rats, and stressed rats. *$p<0.05$.

To identify the subtype of α_1 adrenoceptors involved in the effects of NE on control rats, we first applied NE (10 μM) in the additional presence of CEC (10 μM) and BMY 7378 (300 nM) to block α_{1B} and α_{1D} adrenoceptors. There was no significant attenuation of the effects of NE in the presence of these antagonists. Thus, NE increased the frequency of sIPSCs from 2.8 ± 2.4 Hz to 27.1 ± 7.9 Hz ($p<0.01$, n = 6), and the amplitude of sIPSCs to 154 ± 11.3% of control values ($p<0.05$, n = 6). Next, we examined the effects of the specific α_{1A} adrenoceptor agonist A61603. In control rats, A61603 (1 μM) increased the frequency and amplitude of sIPSC to 1034 ± 158.6 % and 162 ± 14.2% of control values, respectively ($p<0.01$, n = 16; Fig. 3A).

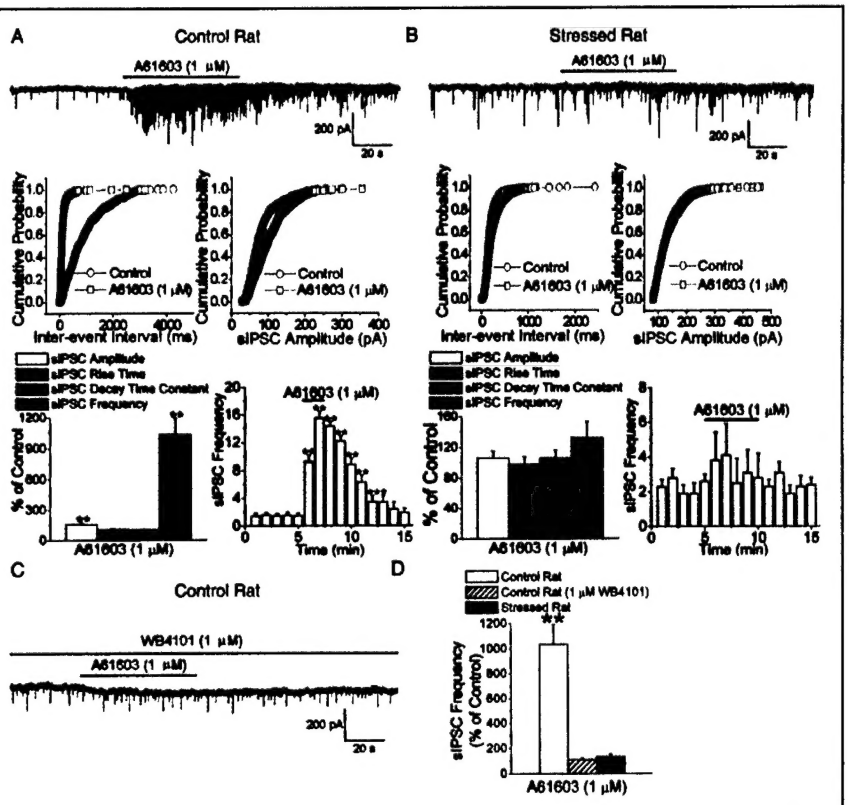


Figure 3. Activation of α_{1A} adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (A) Top trace: Effects of A61603 on spontaneous IPSCs recorded from a BLA pyramidal cell of a control rat. (B) Top trace: sIPSCs recorded from a BLA pyramidal cell of a stressed rat. Middle graphs: Cumulative probability plots of inter-event intervals and amplitude of sIPSC. Bottom graphs: (C) WB4101 prevented the NE-induced increase of sIPSCs. (D) Bar graph shows the effects of A61603 on the mean frequency of sIPSCs recorded from control rats, and stressed rats. *$p<0.05$.

There were no effects on the rise time or decay time constant of sIPSCs (Fig. 3A). In stressed rats, A61603 had no significant effect (Fig. 3B). Thus, in the presence of 1 μM A61603 the frequency of sIPSCs was 132 ± 21% and the amplitude of sIPSCs was 106 ± 8.8% of control values (n = 18, Fig. 3B). The effects of A61603 on sIPSCs in control rats were blocked by the selective α_{1A} adrenoceptor antagonist WB4101 (1 μM, Fig.3C and D).

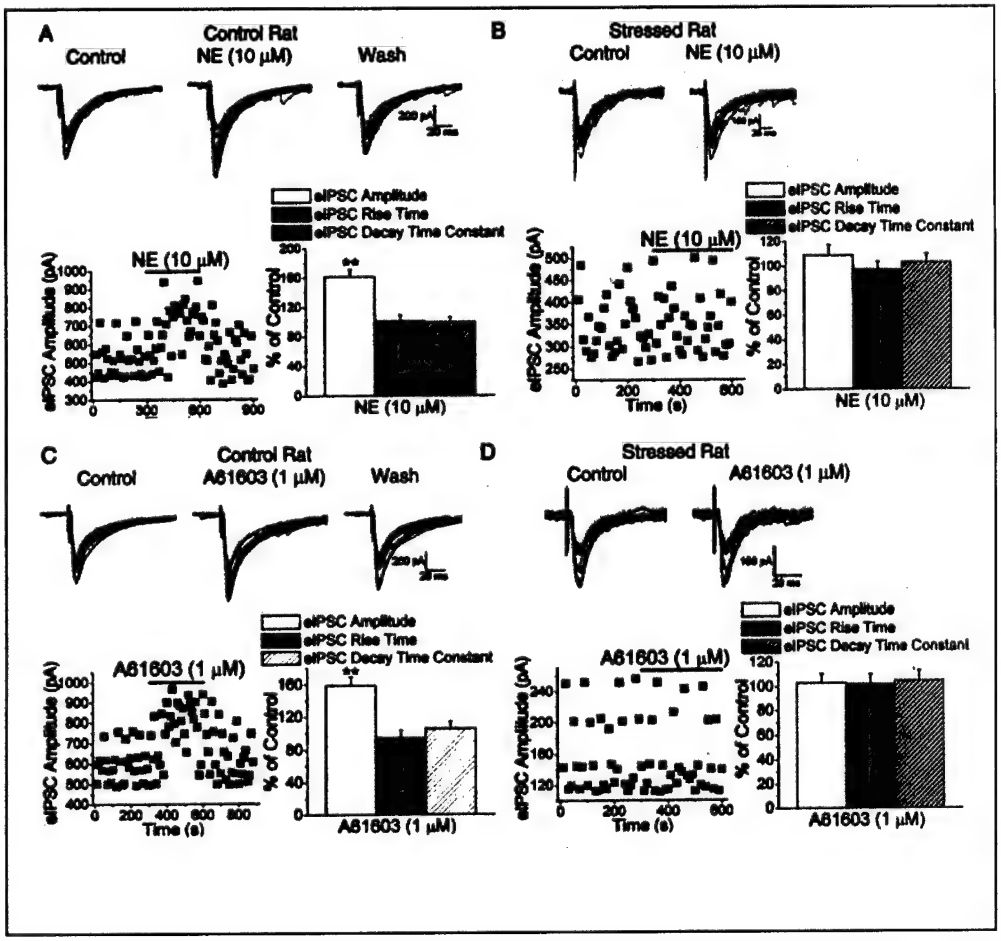
Taken together these results suggest that NE enhances tonic inhibition of pyramidal cells in the BLA, by inducing a massive increase in action potential-dependent spontaneous release of GABA via α_{1A} adrenoceptors, and stress impairs this function of α_{1A} adrenoceptors.

Noradrenergic modulation of evoked IPSCs

It has been shown previously that NE reduces evoked inhibitory transmission in the hippocampus via α adrenoceptors. More recently, in the sensorimotor cortex, it was found that NE actually has a small facilitatory effect on evoked IPSCs, which is detected when $GABA_B$ receptors are blocked. To determine the effects of NE on evoked inhibitory

transmission in the BLA we applied 10 μM NE while recording evoked IPSCs (eIPSCs) in control rats. In the absence of a $GABA_B$ receptor antagonist, NE (10 μM) reduced the amplitude of eIPSCs to 48.2 ± 10.3% of control levels ($p<0.01$, n=8). However, in the presence of SCH50911 (20 μM), a specific antagonist of the $GABA_B$ receptors, NE enhanced the amplitude of eIPSCs to 162.4 ± 9.3% of control, $p<0.01$, n = 10; Fig. 4A) without affecting the rise time and decay time constant of the eIPSCs (Fig. 4A). Similar effects were obtained when α_{1A} adrenoceptors were activated by application of 1 μM A61603 (Fig 4C). Thus, A61603 (1 μM) increased the amplitude of eIPSCs to 159.4 ± 10.7% of control ($p<0.01$, n = 8, Fig. 4C) without affecting the kinetics of the eIPSCs (Fig. 4C). The effects of the drugs were reversible. In stressed rats, neither NE nor A61603 had a significant effect on the amplitude, rise time and decay time constant of eIPSCs (Fig. 4B and D). In the presence of NE (10 μM), eIPSC amplitude was 109 ± 8.2% of the control (n = 11), and in the presence of A61603 the amplitude of the evoked IPSCs was 103 ± 7.4 % of the control (n = 10). These results suggest that 1) NE facilitates evoked GABAergic transmission via α_{1A} adrenergic receptors, 2) this facilitatory effect is masked due to activation of presynaptic $GABA_B$ autoreceptors following the NE-induced enhancement of spontaneous GABA release, and 3) stress blocks the facilitatory effect of NE on evoked GABA release.

Figure 4. In the presence of a $GABA_B$ receptor antagonist, activation of α_{1A} adrenoceptors increases the amplitude of evoked IPSCs in control rats, but not in stressed rats.



(A) Top traces: Evoked IPSCs (eIPSCs) recorded from a BLA pyramidal cell of a control rat. In addition to D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and yohimbine (20 μM), the slice medium also contains 20 μM SCH50911. Norepinephrine increased the amplitude of the eIPSCs, without affecting their kinetics. Bottom graphs: The plot shows the time-course of the NE effect on eIPSC amplitude (same cell as in the top traces). The bar graph shows the effect of NE on the amplitude and kinetics of eIPSCs. (B) Data similar to those shown in (A), but from stressed rats. (C) In control rats, the α_{1A} agonist A61603 produced similar effects to those of NE. Top traces and bottom left plot show data from the same cell. (D) In stressed rats, A61603 had no significant effects on eIPSCs.

Noradrenergic modulation of mIPSCs

The enhancement of eIPSCs and action-potential dependent sIPSCs by NE could be due to a depolarizing effect via activation of somatodendritic α_{1A} adrenoceptors on GABAergic neurons, and/or due to a direct effect at GABAergic terminals. To determine whether, in the BLA, NE modulates GABA release by a direct effect on GABAergic terminals, we tested the effects of NE on miniature IPSCs (mIPSCs), which do not depend on presynaptic invasion of action potentials or Ca^{2+} influx. Miniature IPSCs were recorded in medium containing D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), yohimbine (20 μM) and TTX (1 μM). In the absence of NE, the frequency of mIPSCs was 0.68 ± 0.32 Hz and their amplitude was 114.0 ± 12 pA (n =10). NE (10 μM) increased the frequency of mIPSCs to 182.3 ± 9.6 % of control ($p<0.01$, n = 10; Fig. 5A). The amplitude, rise time and decay time constant of the mIPSCs were not significantly affected by 10 μM NE (Fig. 5A). Similar effects were observed after application of the α_{1A} specific agonist A61603 (Fig. 5C). A61603 (1 μM) increased the frequency of mIPSCs from 0.71 ±0.24 to 1.28 ± 0.31 (178 ± 12.4% of control, $p<0.01$, n = 9; Fig. 5C). The amplitude and kinetics of mIPSCs were not affected by A61603 (Fig 5C). In stressed rats, neither NE (10 μM) nor A61603 (1 μM) produced a significant effect on mIPSCs frequency, amplitude or kinetics (Fig. 5B and C). Thus, the frequency of mIPSCs was 0.68 ± 0.25 Hz and 0.64 ± 0.34 Hz before and after application of NE, respectively (n = 10), and 0.72 ± 0.27 Hz and 0.63 ± 0.31 Hz in the presence and absence of 1 μM A61603, respectively (n = 8). These results suggest that 1) NE facilitates GABA release by a direct effect on GABAergic terminals, and 2) this mechanism of noradrenergic facilitation of GABA release is impaired by stress.

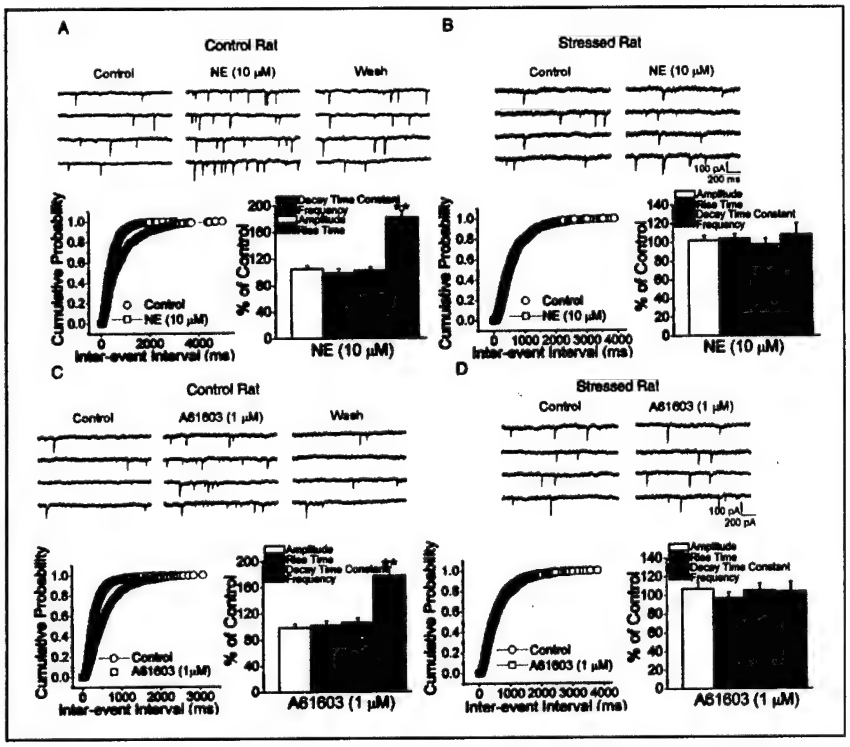


Figure 5. Activation of α_{1A} adrenoceptors increases the frequency of mIPSCs in control rats, but not in stressed rats. (A) Top traces: mIPSCs recorded from a BLA pyramidal neuron of a control rat. NE (10 uM) increased the frequency of mIPSCs. Bottom graph: Left panel shows the cumulative probability plots of inter-event intervals of mIPSCs in control conditions and during application of NE. Bar graph shows the effect of NE on the amplitude, kinetics, and frequency of mIPSCs **$p<0.01$. (B) Similar data to those shown in (A), but from stressed rats. NE had no significant effect on mIPSCs. (C) In control rats, the α_{1A} antagonist A61603 had similar effects to those induced by NE. (D) A61603 had no significant effects on mIPSCs recorded from BLA pyramidal cells of stressed rats. Bar graphs show pooled data from 8 cells.

Facilitation of GABAergic transmission by α_{1A} adrenoceptors is mediated by phospholipase C.

Studies in other brain regions or cell types have shown that α_1 adrenoceptors are coupled to phospholipase C (PLC) via a G-protein, and can increase intracellular calcium by

mobilizing Ca^{2+} from intracellular stores, as well as by increasing Ca^{2+} influx. However, certain effects of α_{1A} activation involve signaling pathways that are independent of PLC activation and intracellular Ca^{2+} rise. To determine whether the α_{1A} adrenoceptor-mediated facilitation of GABA release in the BLA involves activation of PLC, we examined whether the effects of NE on GABAergic transmission are blocked by a PLC inhibitor. In control rats, NE (10 μM) or A61603 (1 μM) enhanced the frequency and amplitude of sIPSCs in the presence of U73343 (20 μM), the inactive isomer of the PLC inhibitor U73122, but had no effects in the presence of 20 μM U73122 (Fig. 6). Thus, in the presence of U73343, NE increased the frequency of sIPSCs to 1022.8 ± 105.3% of control levels ($p<0.01$, n = 8; Fig. 6) and increased the amplitude of sIPSCs to 161 ± 11.7% of control levels ($p<0.01$, n = 6; Fig 6). A61603 (1 μM) increased the frequency of sIPSCs to 978.1 ± 102.1% ($p<0.01$, n = 8; Fig. 6), and increased the amplitude of sIPSCs to154 ± 12.3% of control levels ($p<0.01$, n = 8; Fig 6). In contrast, in the presence of U73122 (20 μM) NE (10 μM) and A61603 (1 μM) failed to induce any significant changes in the frequency and amplitude of sIPSCs . Similarly, the effects of NE (10 μM) on the amplitude of eIPSCs, as well as on the frequency of mIPSCs were blocked by 20 μM U73122 (not shown).

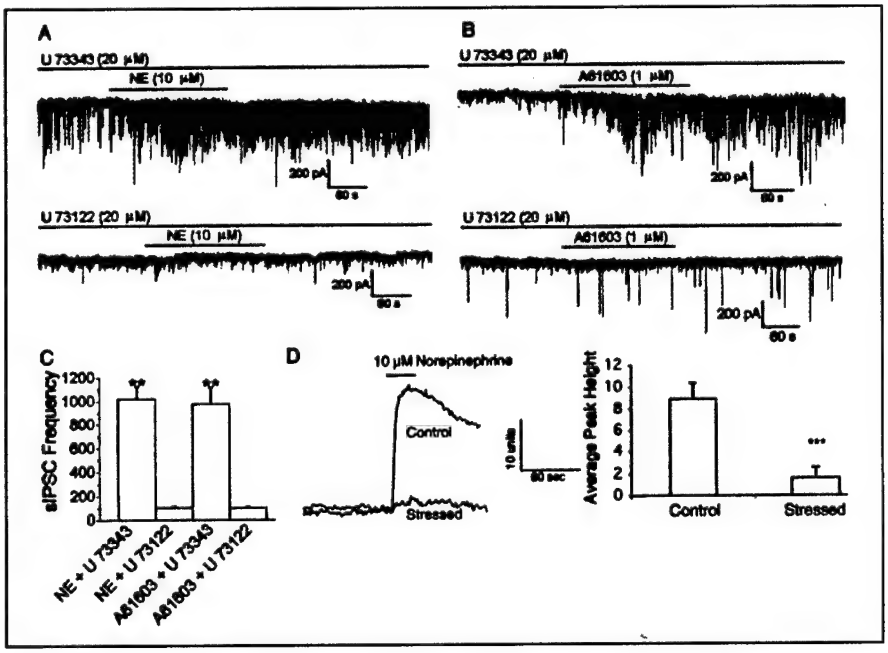


Figure 6. α_{1A} adrenoceptors in the BLA are coupled to phospholipase C. (A) and (B). Spontaneous IPSCs (sIPSCs) recorded from BLA pyramidal neurons. NE (A) or A61603 (B) increased the frequency and amplitude of sIPSCs in the presence of the inactive isomer of a PLC inhibitor (U73343), but had no effect in the presence of the PLC inhibitor U73122. The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and yohimbine (20 μM). (C) Bar graphs showing the effects of NE (10 μM) or A61603 (1 μM) on the frequency of sIPSCs, in the presence of U73343 or U73122. Pooled data from 8 neurons.

Next, we examined whether activation of α_{1A} adrenoceptors by NE, in the BLA, enhances the concentration of intracellular Ca^{2+}. In Fura-2AM-loaded slices, 10 μM NE was applied for 30 s, in the presence of bicuculline (10 μM), SCH50911 (5 μM), CNQX (50 μM), D-AP5 (50 μM), propranolol (10 μM), and yohimbine (20 μM). Intracellular Ca^{2+} concentration was significantly enhanced in slices from control rats, whereas NE had no significant effect in slices from stressed rats (Fig. 6D). Thus, in control rats, NE (10 μM) produced a 4.21 ± 1.47-fold change relative to baseline (change in ratios of fluorescence intensity, $p<0.01$, n = 14; Fig. 6D), while in stressed rats there was only a 1.70 ± 0.94-fold change (n = 14; Fig. 6D). Similar results were obtained when α_{1A} adrenoceptors were activated by application of A61603 (1 μM). In control rats, A61603 (1 μM) induced a significant increase in intracellular Ca^{2+} (4.68 ± 0.17-fold change relative to baseline, $p<0.05$,

n = 16), while in the stressed rats A61603 failed to cause a significant enhancement (0.198 ± 0.081-fold change, n = 16).

Stress blocks α_{1A} adrenoceptor-mediated suppression of BLA field potentials.

Since activation of α_{1A} adrenoceptors facilitates GABAergic transmission, the function of these receptors at the network level could be to dampen neuronal excitability and responsiveness. However, while spontaneous GABAergic activity is dramatically enhanced by activation of α_{1A} adrenoceptors (Fig. 2), evoked GABAergic transmission is suppressed due to presynaptic inhibition of GABA release via $GABA_B$ autoreceptors (Fig. 3). Therefore, under physiological conditions, when $GABA_B$ receptors are not blocked, α_{1A} adrenoceptor activation could enhance amygdala's responsiveness (due to the reduction in evoked GABA release), unless the enhancement of spontaneously released extracellular GABA plays a more decisive role on neuronal excitability. To determine the net effect of α_{1A} adrenoceptor activation on neuronal responsiveness and excitability in the BLA, and whether this effect is altered by stress, we investigated the effects of NE or A61603 on population field responses, in the absence of $GABA_B$ receptor blockade, in control and stressed rats.

Field potentials in the BLA were evoked by stimulation of the external capsule. These responses consist of one major negative component that corresponds in time course to the EPSP recorded intracellularly from BLA pyramidal cells, and is mediated by AMPA/kainate receptors (Aroniadou-Anderjaska et al., 2001). In control rats, 10 μM NE, in the presence of propranolol (10 μM) and yohimbine (20 μM), produced a significant reduction in the peak amplitude of evoked field potentials (83.8 ± 5.3% of control levels, n = 14, $p<0.05$; Fig. 7A). Similarly, bath application of 1 μM A61603 caused a significant reduction in peak amplitude of the field potentials to 83.1 ± 5.2% of control levels ($p<0.05$, n = 12; Fig. 7B). In contrast, in stressed rats, neither NE (10 μM) nor A61603 (1 μM) had a significant effect on the amplitude of the field potentials (Fig. 7). These results suggest that the function of α_{1A} adrenoceptors in the BLA is to reduce neuronal excitability/responsiveness and that this function is impaired by stress.

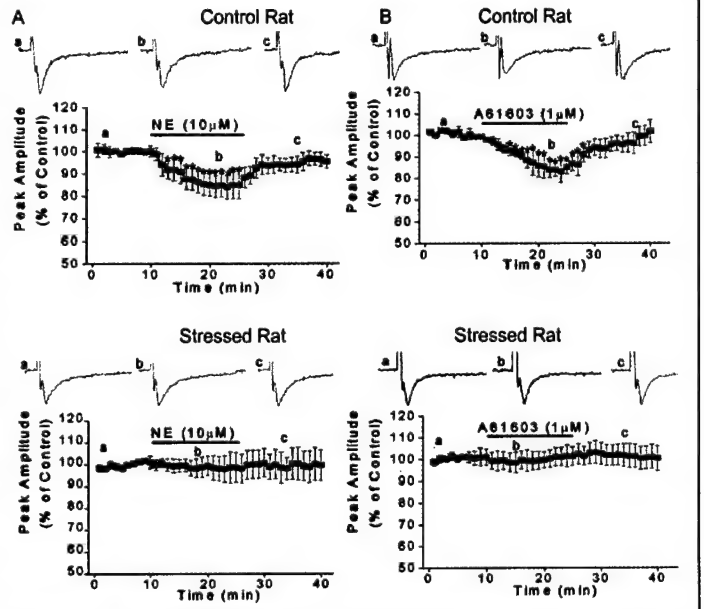


Figure 7. Activation of α_{1A} adrenoceptors reduces BLA field potentials in control rats, but not in stressed rats. (A) Changes in the peak amplitude of BLA field potentials evoked by stimulation of the external capsule, in response to bath application of 10 μM NE, in control (top panel, n = 9) and stressed (bottom panel, n = 10) rats. The medium contains propranolol (10 μM) and yohimbine (20 μM). (B) Similar data to those in (A), except that A61603 is applied in place of the NE. Pooled data from 10 slices (control rats, top panel) and 8 slices (stressed rats, bottom panel). Slice medium same as in (A). Asterisks over error bars denote statistically significant reduction ($p< 0.05$).

Stress blocks the α_{1A} adrenoceptor-mediated modulation of Long-Term Potentiation.

The memories of traumatic, stressful events are thought to be stored in neocortical areas, with the amygdala playing a major role in modulating the consolidation of these memories. Furthermore, the amygdala itself is thought to be the site of memory formation during stressful experiences that are similar to fear-conditioning. At the cellular level, memory traces are believed to be formed by enduring strenthening of neuronal synapses, within specific neuronal networks. Changes in synaptic strength are expressed as changes in the efficacy of synaptic transmission, such as Long-Term Potentiation (LTP). Both fear-conditioning and other types of stressors are associated with changes in the efficacy of synaptic transmission in the amygdala. Using our stress model, we have found that stress alters the noradrenergic modulation of LTP in the BLA in a manner that facilitates the induction of LTP. Thus, in control rats, theta-burst stimulation (TBS) induces an NMDA receptor-dependent, input-specific LTP of the BLA field potential evoked by stimulation of the external capsule (Fig. 8).

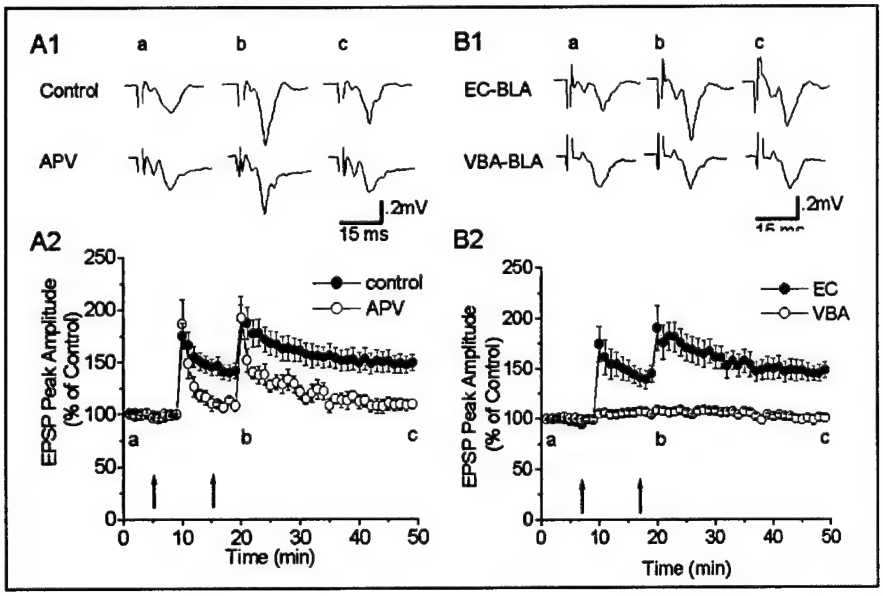


Figure 8. Two theta-burst stimulations (TBS, interval=10 min) of EC induce LTP in the BLA. A: LTP is induced by two TBS of EC (filled circles) and is blocked by NMDA receptor antagonist APV (100 μM, open circles). The peak amplitude of EPSPs 30 min after the second TBS was 149.4±7.9% (n=10) in control and 109.4±4.6% (n=5) for field potentials in the presence of APV. **B:** Two TBS (interval=10 min) of EC induced-LTP (filled circles) is not associated with any change in responsiveness to BA stimulation (open circles, without TBS in VBA-BLA pathway). The peak amplitude of EPSPs 30 min after the second TBS was 148.5±7.9% (n=5) in EC-BLA while in VBA-BLA pathway the EPSPs remained 100.3±2.9% (n=5) of baseline values. The values are expressed as a percentage of the mean of 60 responses at 0.1 Hz before the application of TBS. Each point represents the mean±SEM. TBS was applied at times shown by arrows. Insets show typical traces of EPSPs at the times indicated by the letters **a**, **b** and **c**. Traces are the averages of 6 consecutive responses to the stimulation at 0.1 Hz.

During simultaneous field potential and intracellular recordings, the time course of LTP of the field potential corresponds to that of the EPSP, as we have observed previously (Aroniadou-Anderjaska et al., 2001).

In the presence of the α_{1A} adrenoceptor agonist A61603, TBS does not induce LTP. A61603 reduces evoked field potentials (Fig. 9). LTP is blocked by A61603 whether TBS is applied to the reduced field potentials or after the stimulation intensity is increased to produce a field potential of the same amplitude as the control (in the absence of A61603). In stressed rats, the presence of A61603 does not prevent the induction of LTP (Fig 9). Thus, the stress-induced impairement in the function of α_{1A} adrenoceptors affects the modulation of LTP in the BLA, in a manner that facilitates the induction of LTP. The stress-induced blockade of the α_{1A} adrenoceptor-mediated suppression of LTP may be one of the mechanisms responsible for the over-consolidation of memories associated with stressful events in PTSD patients.

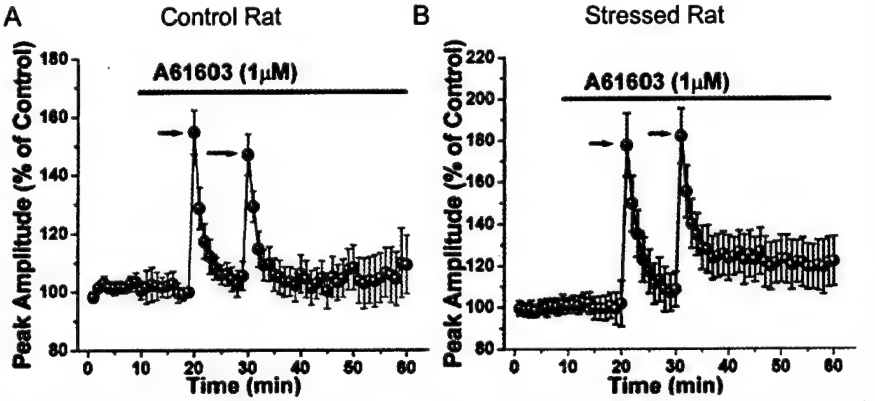


Figure 9. A: Activation of α_{1A} adrenoceptors blocks the induction of LTP. Group data from 12 animals. Error bars are standard errors. **B:** Stress blocks the α_{1A} adrenoceptor-mediated inhibition of LTP. Group data from 14 animals. Error bars are standard errors.

The effects of α_2 adrenoceptor activation on the norepinephrene-mediated modulation of GABAergic synaptic transmission in the basolateral amygdala neurons of control and stressed rats

By means of the whole-cell patch clamp technique, we have examined the hypothesis that the noradrenergic modulation of GABAergic transmission in the BLA is also mediated via activation of α_2 adrenoceptors.

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded from BLA pyramidal neurons at a holding potential of –70 mV, and in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and prazosin (1 μM). In control rats, the mean frequency of sIPSCs recorded in the soma of BLA pyramidal neurons was 3.6 ± 2.2 Hz. Bath application of bicuculline (10 μM) eliminated sIPSCs, confirming that they were mediated by $GABA_A$ receptors. NE (10 μM) caused a significant decrease of the mean sIPSC frequency that persisted throughout the application of NE and was completely reversed after removal of the agonist (Fig. 10). These effects of NE were not accompanied by any significant change in the rise time or decay time constant of sIPSCs, and were blocked by the α_2 adrenoceptor antagonist yohimbine (20 μM), confirming that NE was acting via α_2 adrenergic receptors. These results indicate that activation of α_2 adrenoceptors modulates the action potential-dependent release of GABA from BLA interneurons and thus, suggest that these receptors may play an important role in the regulation of the overall excitability of this brain region.

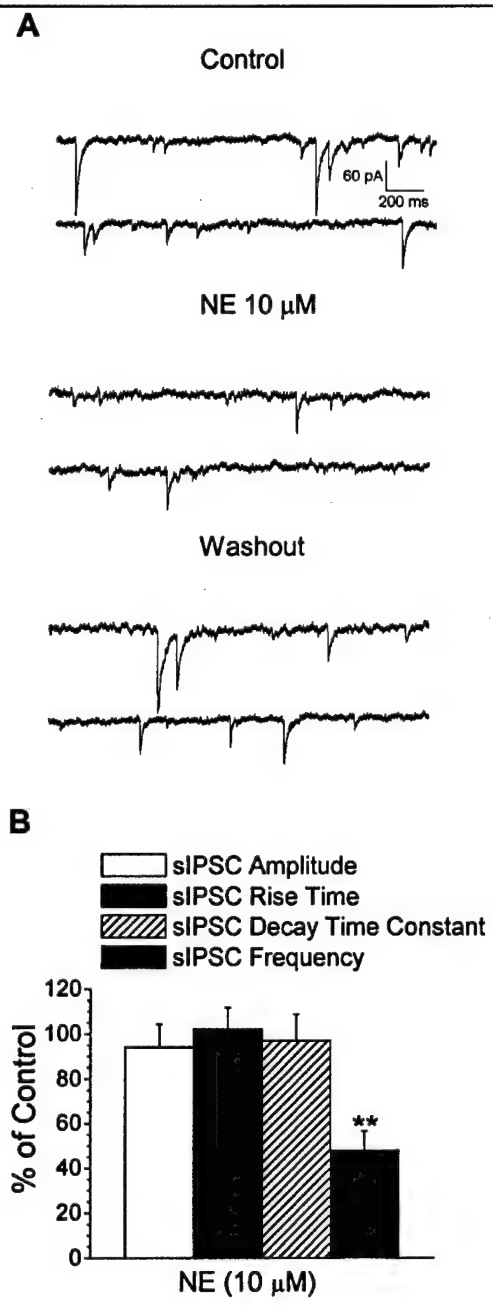


Figure 10. Activation of α_2 adrenoceptors reduces tonic inhibition of BLA pyramidal neurons. **(A)** Top trace: Effects of NE (10 μM) on spontaneous IPSCs (sIPSCs) recorded from a BLA pyramidal cell. Holding potential is -70 mV. The medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and prazosin (1 μM). **(B)** Bottom graph: Pooled data (mean ± SEM) from 8 neurons. Bar graph shows the NE-induced changes in amplitude, frequency and kinetics of sIPSCs. **$p<0.01$.

We have also investigated whether NE is able to modulate evoked inhibitory transmission in the basolateral amygdala via activation of α_2 adrenoceptors. To determine the effects of NE on evoked inhibitory transmission in the BLA, we applied 10 μM NE while recording evoked IPSCs (eIPSCs). In the presence of SCH50911 (20 μM), a specific antagonist of $GABA_B$ receptors, NE significantly reduced the amplitude of evoked IPSCs without affecting the rise time and decay time constant of the evoked IPSCs (Fig. 11A). These effects were mediated via the activation of α_2 adrenoceptors because they were blocked by

yohimbine (20 μM, Fig. 11).

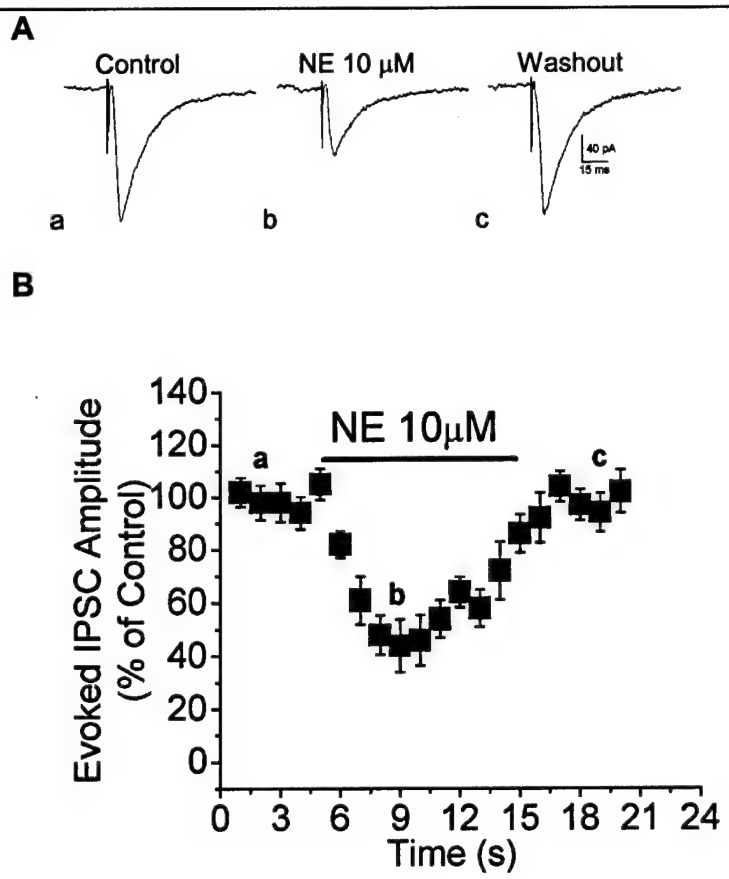


Figure 11. In the presence of a $GABA_B$ receptor antagonist, activation of α_2 adrenoceptors reduces the amplitude of evoked IPSCs. (A) Top traces: Evoked IPSCs (eIPSCs) recorded from a BLA pyramidal cell. In addition to D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM) and prazosin (10 μM), the slice medium also contains 20 μM SCH50911. Norepinephrine (10 μM) reduced the amplitude of the evoked IPSCs, without affecting their kinetics. (B) Bottom graph: The plot shows the time-course of the NE effect on evoked IPSC amplitude pooled from 10 neurons. **p<0.01.

The role of β adrenergic receptors on the synaptic function, neuroplasticity and calcium signaling patterns in basolateral amygdala neurons in control and stressed rats in vitro.

We have investigated the role of isoproterenol, a β adrenoceptor agonist, on BLA synaptic transmission, neuroplasticity and calcium signaling by using field and intracellular recordings and calcium imaging. NMDA, AMPA, and $GABA_A$ and $GABA_B$ receptor mediated synaptic potentials were blocked by inclusion of 100 μM (-)-2-amino-5-phosphonopentanoic acid, 10 μM bicuculline, 10 μM SCH 50911 and 50 μM GYKI 52466 or GYKI 53655 in the perfusion solution. The effect of β-adrenergic receptor stimulation on intracellular calcium signaling in the BLA neurons was also measured by fluorescence microphotometry with the membrane permeable calcium-specific dye calcium green.

Calcium Measurement

Amygdala slices were incubated with ACSF containing 15 μM fura-2 acetoxymethyl ester (Fura-2 AM) and 0.02% Pluronic F127 at 37° C for 30 min and then rinsed in ACSF at room temperature for an additional 15-30 min to remove un-incorporated Fura-2 AM. The slices were transferred to a chamber mounted on an upright Zeiss microscope, then submerged and superfused with ACSF at 2ml/min at room temperature. The microscope was coupled to a DeltaRam monochromator (PTI, Monmouth Junction, NJ) and excitation wavelengths were set to 340 nm and 380 nm. Emitted fluorescence images at 510 nm or higher were captured at a rate of 2 Hz through a 63x Zeiss water immersion objective (N.A. 0.95) with a digital CCD camera (ORCA 100, Hamamatsu, Tokyo, JP) and collected using OpenLab imaging software from Improvision (Lexington, MA). The ratio of emission fluorescence intensity of neuronal somata at 340 nm and 380 nm were calculated with the Improvision software. Peak heights were defined as the difference between the maximum fluorescence ratio value of the smoothed peak and the baseline averaged over 10 s immediately preceding the peak.

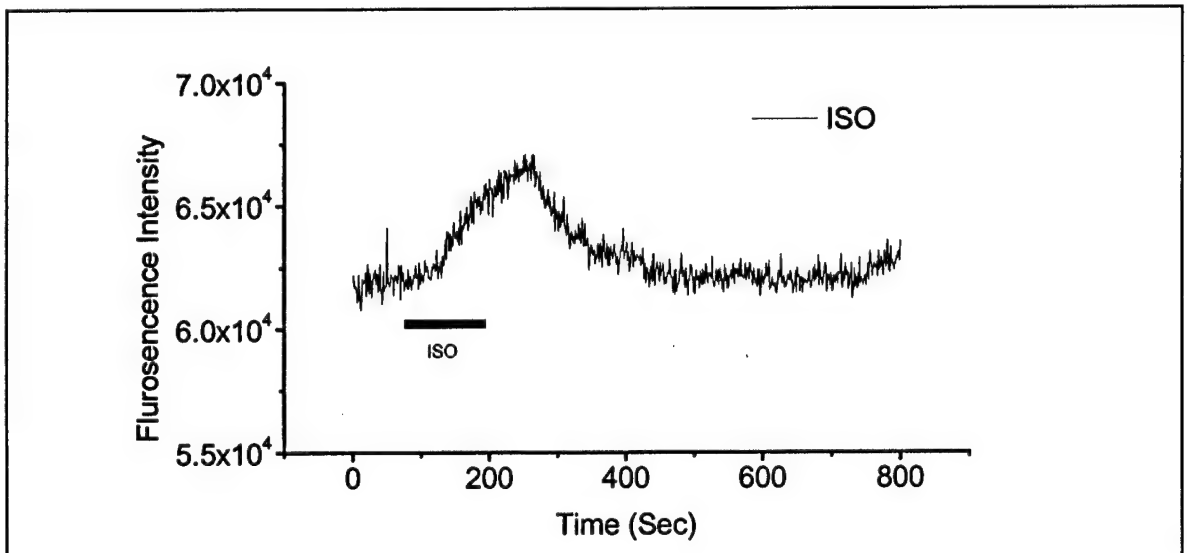


Figure 12. Isoproterenol, a β receptor agonist, enhanced intracellular calcium signal in the basolateral amygdala circuitry.

In control rats, external capsule (EC) stimulation evoked GluR5 mediated synaptic responses in BLA neurons that was enhanced by application of the β-adrenergic receptor agonist, isoproterenol (10 μM; 116 ± 12 %) and the synaptic response enhanced by isoproterenol were largely blocked by the AMPA/GluR5 kainate receptor antagonist LY377770 (20 μM; 88 ± 5%) or by the GluR5-selective kainate receptor antagonist LY382884B (100 μM; 86 ± 6%, Fig 13 and 14). In addition, isoproterenol elicited an increase of 10315 ± 2609 (n=16) in the fluorescence intensity equivalent to 21% of the response to 50 mM KCl, indicating an increase in intracellular calcium levels in BLA neurons. These results suggest that enhancement of GluR5 kainate mediated synaptic responses takes place through a β-adrenoceptor elicited intracellular calcium increase in BLA neurons and thus may contribute to the impact of stress on synaptic transmission and synaptic plasticity in the amygdala. We have also examined the effects of isoproterenol (10 μM) on action-potential independent, miniature inhibitory postsynaptic currents (mIPSCs) recorded from BLA pyramidal neurons in control rats. mIPSCs were recorded at a holding potential of –70 mV,

and in the presence of TTX (1 μM), D-AP5 (50 μM), CNQX (10 μM), and prazosin (1 μM). Application of isoproterenol (10 μM) caused a significant increase of the mean sIPSC frequency that persisted throughout the application of the drug and was completely reversed after removal of the agonist.

β-adrenergic modulation of GluR5 kainate receptor mediated EPSPs

As initially proposed, we investigated the effects of the β-adrenergic receptor agonist isoproterenol on excitatory synaptic transmission in the basolateral amygdala (BLA). Intracellular recorded excitatory postsynaptic potentials (EPSP) and field excitatory potentials (fEPSP) were evoked by stimulation of the external capsule (EC). GluR5 mediated synaptic responses in BLA neurons were enhanced by application of the β-adrenergic receptor agonist, isoproterenol (10μM; 116 ± 12) and the synaptic response enhanced by isoproterenol was largely blocked by the AMPA/GluR5 kainate receptor antagonist LY377770 (20μM; 88 ± 5%) or by the GluR5-selective kainate receptor antagonist LY382884B (100μM; 86 ± 6%). These results indicate that the activation of β-adrenergic receptors is able to facilitate GluR5 kainate receptor mediated excitatory synaptic transmission in the neuronal circuitry of the basolateral amygdala. More importantly, this mechanism may be involved in the impact of stress on neuronal communication in the amygdala and could play a role in post-traumatic stress disorder (PTSD).

We have also investigated whether β-adrenergic receptors played a role in the mechanisms underlying low frequency train-induced long term potentiation in the amygdala. In other words, we tested the hypothesis that during low frequency stimulation these receptors are activated by norepinephrine, which is co-released with glutamate, contributing to the development of enhanced synaptic efficacy. However, bath application of the β-adrenergic receptor antagonists, timolol (20 μM) or propranolol (10 μM) was unable to block the low frequency stimulation-induced synaptic facilitation (Figure 15). When taken together our results suggest that β-adrenergic receptor activation modulates excitatory synaptic pathways in the basolateral amygdala preferentially via facilitation of tonic synaptic neurotransmission rather than by activity-dependent neuronal mechanisms. The involvement of intracellular second messenger systems in the long term potentiation induced by low frequency stimulation will be further examined by using a specific protein kinase A inhibitor in the amygdala slice preparation.

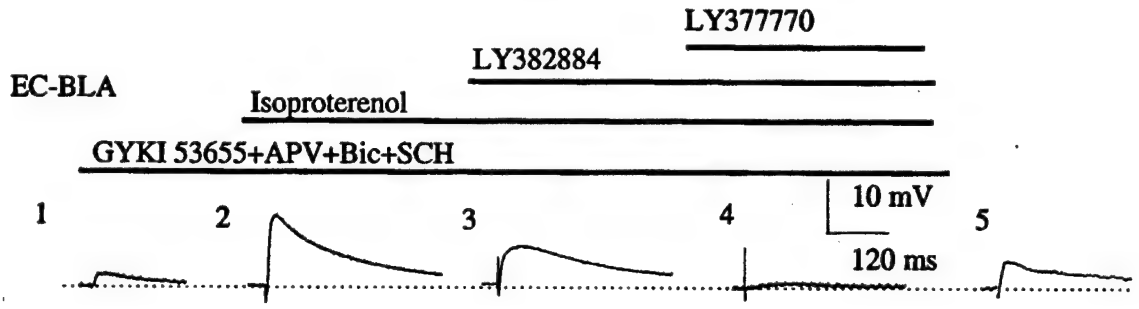


Figure 13. β adrenergic activation enhances GluR5 kainate receptor mediated synaptic potentials in the basolateral amygdala. Shows the average of intracellular recordings of the EC-BLA pathway after addition of GYKI 53655(50 μM) , APV (100 μM), bicuculline (10 μM), and SCH50911 (10 μM, 1), after addition of isoproterenol (2), after addition of LY382884B (3), after the addition of LY377770 (4), and after a washing out period (5).

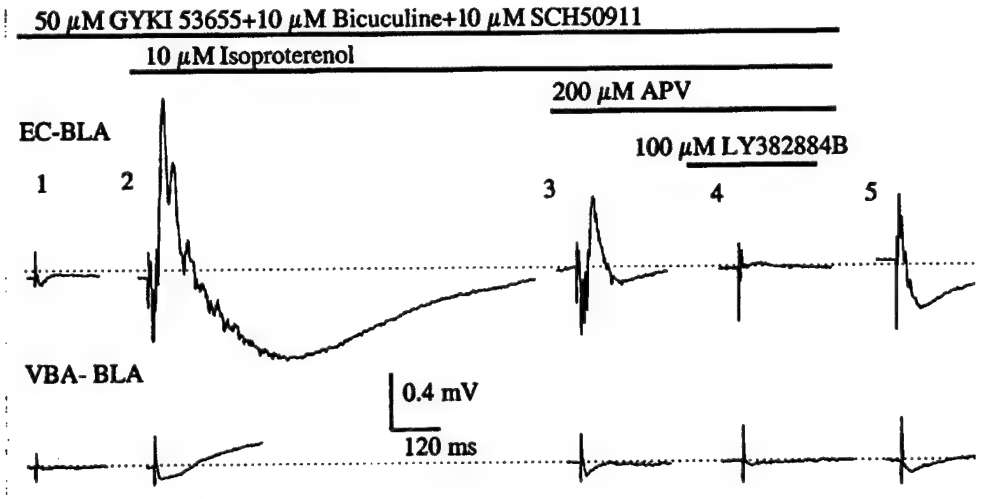


Figure 14. β adrenergic activation enhances GluR5 kainate receptor mediated synaptic potentials in the basolateral amygdala. Shows the average of field recordings of the EC-BLA pathway after addition of GYKI 53655(50 μM), bicuculline (10 μM), and SCH50911 (10 μM, 1), after addition of isoproterenol (2), after addition of APV (3), after the addition of LY382884B (4), and after a washing out period (5).

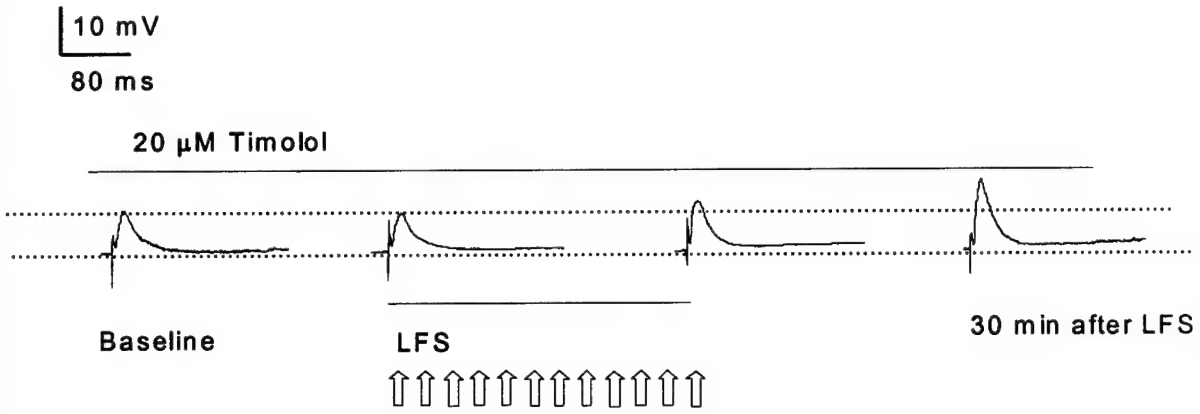


Figure 15. In the presence of timolol, a β adrenergic receptor antagonist, low frequency stimulation remains capable of inducing long term potentiation in the EC and in BLA synaptic transmission.

We have also investigated the effects of stress on the function of β adrenoceptors expressed in amygdala neurons. The effect of β adrenergic receptor stimulation on intracellular calcium signaling in the BLA neurons of stressed rats was measured by fluorescence microphotometry with the membrane permeable calcium-specific dye calcium green. In contrast to the down-regulation/desensitization of α_1-adrenergic receptors observed after three days of stress, we have observed that the β-adrenergic responses to isoproterenol (these manifest a significant increase in intracellular calcium, as reported in a previous annual report) appear to be up-regulated by stress when tested on the day of or day after the stress. These findings may be very important for understanding what happens immediately after a stressful experience that leads to PTSD.

Stress impairs 5-HT_2 receptor-mediated facilitating effects on synaptic plasticity in the rat basolateral amygdala

Stress-related affective disorders are often associated with changes in emotional learning and memory, implicating possible dysfunction of the neuronal plasticity. Enhanced serotonin release during periods of anxiety and stress has been observed in the amygdala complex (Fernandes et al., 1994). The amygdala expresses high levels of 5-HT_2 receptor protein and mRNAs (Morilak et al., 1994;Pompeiano et al., 1994;Wright et al., 1995). Activation of 5-HT_2 receptors in the amygdala facilitates activity-dependent neuroplasticity in the amygdala circuitry (Wada et al., 1997) and has a profound affect upon anxiety and mood (Hrdina et al., 1993;Kshama et al., 1990;Tokuyama et al., 1993). Therefore, understanding the changes in amygdala neuroplasticity and how its underlying cellular mechanisms are affected by stress is critical in understanding the pathophysiology of stress, and may aid in the development of new therapeutic strategies for the prevention and treatment of stress-related affective disorders.

The amygdala complex is well known for its involvement in mood and emotion (Davis, 1992;Ledoux, 1995), and may participate in the pathogenesis of schizophrenia (Bogerts et al., 1985), depression (Drevets et al., 1992), epilepsy (Boucsein et al., 2001;Pitkanen et al., 1998) and posttraumatic stress disorder (Liberzon et al., 1999;Post et al., 1998;Rauch et al., 2000). In these illnesses, the importance of serotonergic neurotransmission is universally acknowledged.

Excitatory neurotransmission in the basolateral amygdala is mediated by NMDA and AMPA/kainate receptors (Li and Rogawski, 1998;Rainnie et al., 1991). This excitatory neurotransmission exhibits NMDA receptor-dependent and receptor-independent long-term synaptic plasticity (Gean et al., 1993;Li et al., 1998;Li et al., 2001;Maren, 1999) The effect of 5-HT2 receptor modulation of synaptic plasticity of amygdala circuitry, however, remains to be elucidated. This form of synaptic plasticity may underlie the learning of traumatic memories that characterize fear conditioning, anxiety disorders and posttraumatic stress disorder.

We have examined the impacts of stress on the facilitative effect of 5-HT2-receptor stimulation on synaptic plasticity in the basolateral amygdala, using intracellular and field potential recording techniques. The results demonstrate that stress impairs the facilitative effect of 5-HT2 receptor activation on the theta burst stimulation induced synaptic plasticity in the basolateral amygdala. Such impairment of synaptic plasticity appears to be part of the cellular mechanism underlying emotional learning disorders observed in major depression and post-traumatic stress disorders.

Theta burst stimulations induce short-term potentiation that is not affected by stress. However, the facilitative effect of DOI, a 5-HT2 receptor agonist, on the theta burst stimulation induced long-term potentiation was impaired after three days of stress.

Single theta-burst stimulation (TBS) induced short-term synaptic potentiation in the basolateral amygdala in both control and stressed rats. Figure 16A shows typical traces (averages of 6 responses) of f-EPSPs that were taken from individual experiments at the times indicated by the letters a, b and c. The slope of f-EPSPs 30 min after TBS was 100.9±8.0% (n=6) in control (filled circles) and 107.8±4.4% (n=6) in stress (open circles). In the presence of DOI, a single TBS induced long-term potentiation (LTP) in control rats but failed to induce LTP in stressed rats (Fig. 16 B). The slope of f-EPSPs was 147.6±1.9% (n=6) in control rats (filled circles) and 98.5±6.7% (n=6) in stressed rats (open circles), respectively, 30 min after the onset of TBS. Each point represents the mean ± SEM. Theta-burst stimulation was applied at times shown by arrows. Insets show typical traces of f-EPSPs at the times indicated by the letters a, b and c. Traces are the averages of 6 consecutive responses to the stimulation at 0.1 Hz.

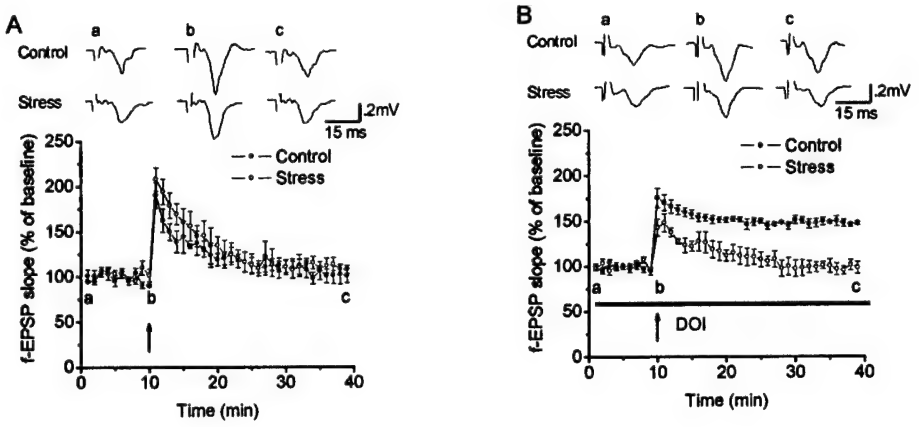


Figure 16. Theta burst stimulation induced short-term potentiation was not affected by stress (1A). However, the facilitative effect of DOI, 5-HT2 receptor agonist, on the inducetion of Long-term potentiation was impaired after stress (1B).

The facilitative effect of DOI on the induction of LTP was blocked in the presence of APV, a NMDA receptor antagonist (Figure 17A). Application of APV, an NMDA receptor antagonist, blocked the facilitative effect of DOI on TBS-induced synaptic plasticity in control rats. The cumulative data were presented as the percentage of f-EPSP slopes (in related to the baseline) in the slices treated with APV (50 μM) and DOI (20 μM). The slope of f-EPSPs 30 min after TBS was 109.2±4.3% (n=7) in control rats in the presence of APV and DOI. Figure 17 shows typical traces (averages of 6 responses) of f-EPSPs that were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows. Bars show the duration of drug application.

The facilitative effect of DOI on NMDA receptor-mediated EPSPs was impaired by stress (Figure 17B). NMDA receptor-mediated EPSPs were enhanced up to 142.9±3.6% (n=7) of the baseline in control rats (filled circles) 15 min after application of DOI. While in the amygdala slices from stressed rats (open circles), the facilitative effect of DOI on NMDA receptor-mediated EPSP was significantly attenuated to 113.0±2.3% (n=7) of the baseline 15 min after the application of DOI. Insets show the typical traces of EPSP at the times indicated by the letters a, b and c.

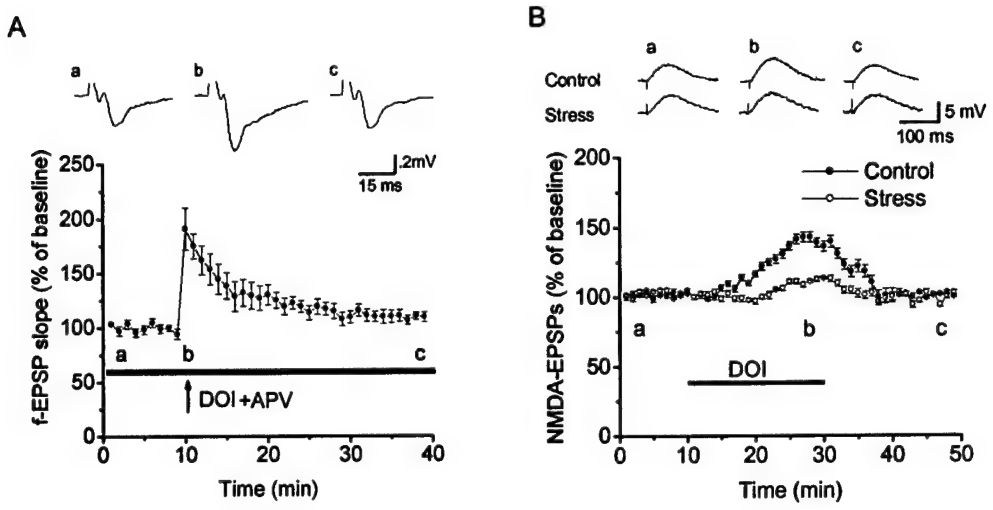


Figure 17. The facilitative effect of DOI on the induction of LTP was blocked in the presence of APV, an NMDA receptor antagonist (2A). The enhancement of NMDA-mediated synaptic potential by DOI was significantly attenuated by stress (2B).

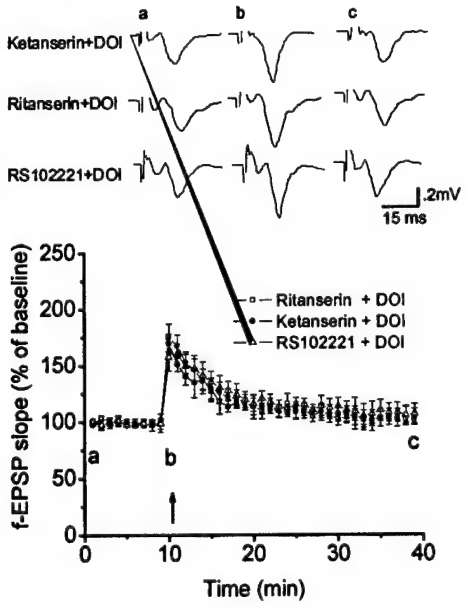


Figure 18. The facilitative effect of DOI on the induction of LTP was abolished in the presence of 5-HT2 receptor antagonists in control rat.

The effects of 5-HT_2 receptor antagonists on the facilitative effect of DOI on TBS-induced synaptic plasticity in control rats are shown in Figure 18. The enhancement of DOI on TBS-induced synaptic potentiation was blocked by the 5-$HT_{2A/2C}$ receptor antagonists ketanserin and ritanserin and by the 5-HT_{2C} receptor antagonist RS 102221 in control rats. The slope of f-EPSPs 30 min after TBS was 106.9±8.7% (n=7) in the presence of ritanserin and DOI, 104.3±7.2% (n=5) in the presence of ketanserin and DOI, 101.4±8.7 (n=5) in the presence of RS 102221 and DOI, respectively. The values are expressed as the percentage of the mean of 60 responses at 0.1 Hz before the application of TBS. Each data point was presented as the mean ± SEM. Theta-burst stimulation was applied at the times indicated by the arrows. The insets show typical traces of f-EPSPs at the times indicated by the letters a, b and c. Each trace is the averages of 6 consecutive responses to the stimulation at 0.1 Hz.

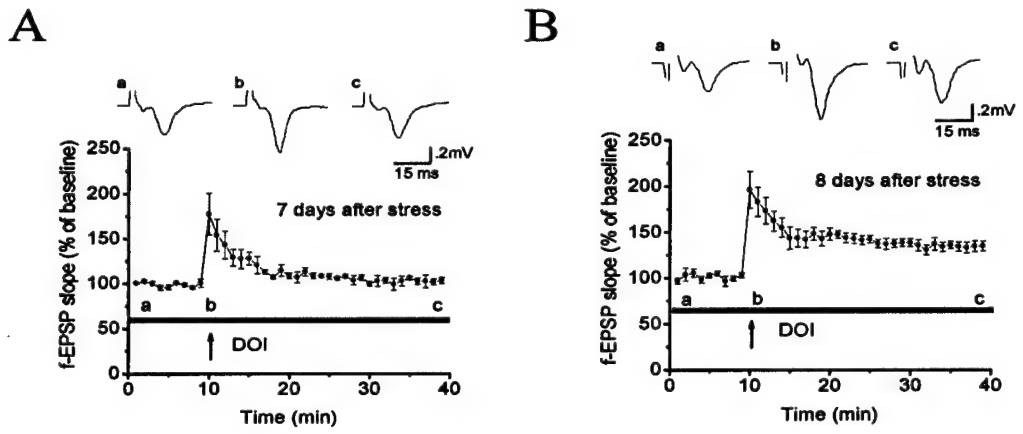


Figure 19. The impairment of 5-HT2 receptor agonist induced facilitative effect on the induction of LTP lasts for 7 days after the termination of the stress paradigm.

Impairment of 5-HT_2 receptor-mediated facilitative effects on TBS-induced LTP lasts for seven days after stress.

The percentage of f-EPSP slopes was measured in relation to baseline in stressed rat BLA seven days after stress (n=5). DOI remains incapable of facilitating TBS-induced synaptic potentiation seven days after stress. The slopes of f-EPSP 30 min after TBS were 103.5±3.5% (n=5) of the baseline (Figure 19A). The typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by a, b and c. TBS was applied at the times indicated by arrows. 19B, The percentage of f-EPSP slopes is presented in relation to baseline in stressed rat BLA eight days after stress (n=7). DOI is again capable of facilitating the effect on TBS-induced synaptic plasticity eight days after stress. The slopes of f-EPSP 30 min after TBS were 134.7±5.3% (n=9) of the baseline. The typical traces (averages of 6 responses) of f-EPSP were taken from individual experiments at the times indicated by a, b and c. The TBS was applied at the times indicated by arrows.

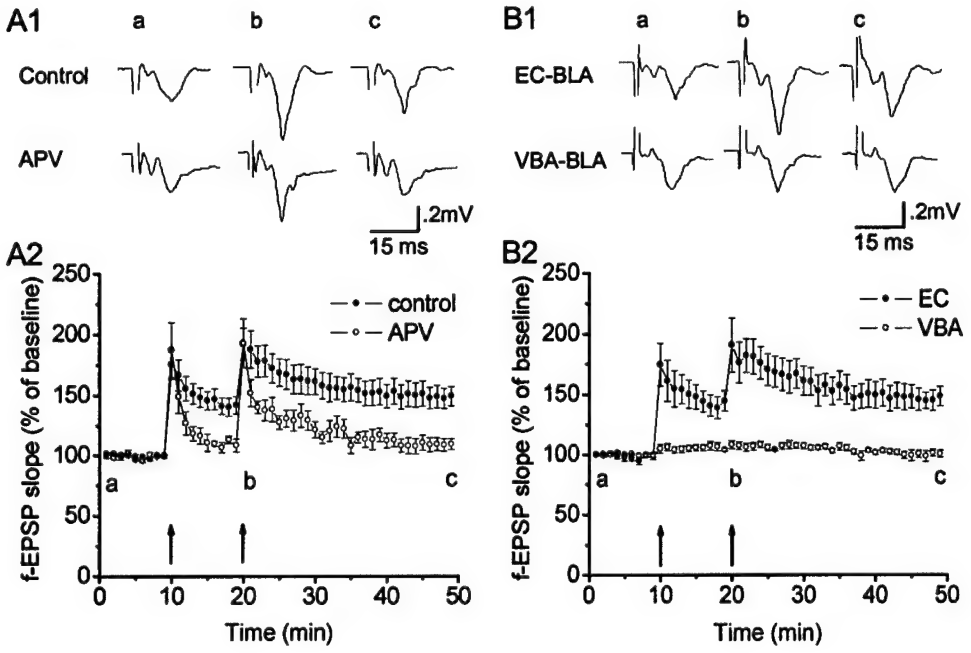


Figure 20. Two theta burst stimulations induce an NMDA receptor-dependent long-term potentiation (A1 and A2), which characterizes a pathway specific synaptic plasticity (B1 and B2).

Two TBS-induced (interval of 10 minutes) LTP is NMDA-dependent and input-pathway specific in BLA.

LTP was induced by two TBS of EC and blocked by the NMDA receptor antagonist APV. Figure 20 A1 shows typical traces (averages of 6 responses) of f-EPSPs at the times indicated by the letters a, b and c in A2. A2, Mean ± SEM percentage of f-EPSP slope (in relation to baseline) in the slices treated with ACSF (black circles, n=10) or 50 μM APV (white circles, n=5). B, Two TBS of EC induced LTP in BLA that was not associated with any change in responsiveness to ventral basal amygdala (VBA) stimulation (no TBS in VBA-BLA pathway). B1, Typical traces (averages of 6 responses) of f-EPSPs at the times indicated by the letters a, b and c in B2. B2, Mean ± SEM percentage of f-EPSP slope (in relation to baseline) in EC-BLA (black circles) or in VBA-BLA (white circles). TBS was applied at the times shown by arrows.

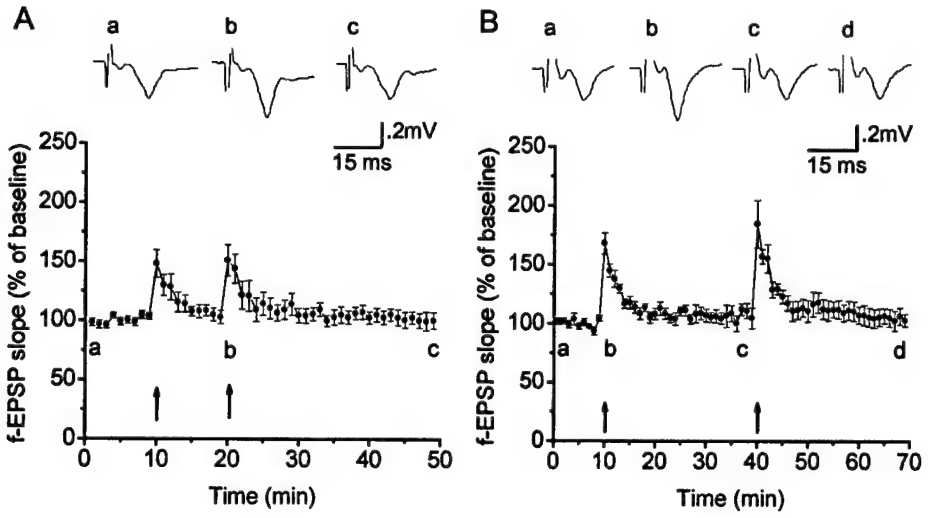


Figure 21. Acute one day stress impairs two theta burst stimulations induced long-term potentiation in the basolateral amygdala immediately after the termination of stress paradigm.

Figure 21 shows that the TBS-induced LTP was impaired after acute (one day) stress. A, Mean ± SEM percentage of f-EPSP slope was presented in relation to baseline in stressed rat BLA (n=7). The interval of two TBS was 10 min. Typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows. B, Mean ± SEM percentage of f-EPSP slope was presented in relation to baseline in stressed rat BLA (n=4). The interval of two TBS was 30 min. Typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows.

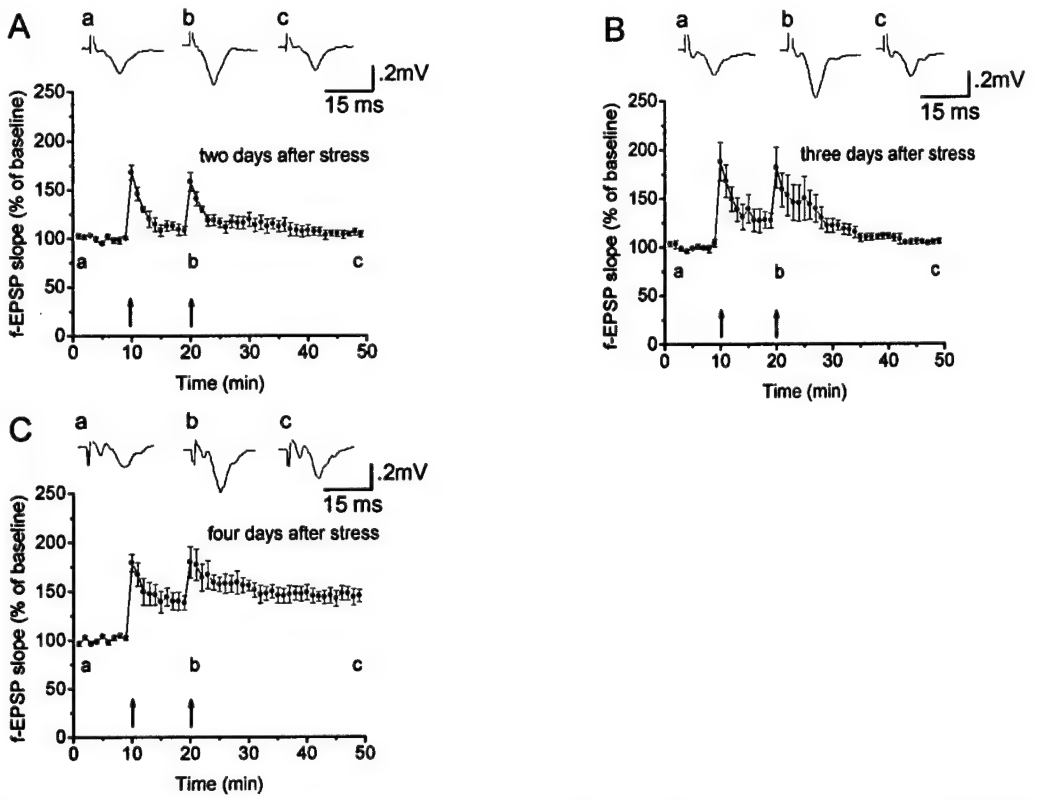


Figure 22. Acute one day stress impairs two theta burst-induced long-term potentiation in the amygdala and the effect of such impairment lasts for three days.

Acute (one day) stress impairs two-theta burst-induced long-term potentiation in the amygdala and the effect of such impairment lasts for three days after the termination of stress paradigm.

In Figure 22A, the Mean ±SEM percentage of f-EPSP slope is presented in relation to baseline in stressed rat BLA two days after stress (n=5). The interval of two TBS was 10 min. Typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows. Two TBS, at an interval of 10 min and at an interval of 30 min, failed to induce LTP. The slopes of f-EPSPs 30 min after the second TBS remained 100.3±6.9% (n=7) at an interval of 10 min and 102.9±5.3% (n=4) at an interval of 30 min of the baseline, respectively. B, Mean ± SEM percentage of f-EPSP slope is presented in relation to baseline in stressed rat BLA three days after termination of stress (n=7). TBS-induced LTP was impaired by three-day stress. The slope of f-EPSPs was 106.5±4.0% (n=5) of the baseline 30 min after the second TBS. The interval between the two TBS was 10 min. Typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows. C, Mean ± SEM percentage of f-EPSP slope was presented in relation to baseline in stressed rat BLA four days after stress (n=7). Two TBS failed to induce LTP until four days after stress (Fig. 7). The slope of f-EPSPs 30 min after TBS was 145.2±6.2% (n=7) of the initial baseline values. The interval between two TBS was 10 min. Typical traces (averages of 6 responses) of f-EPSPs were taken from individual experiments at the times indicated by the letters a, b and c. TBS was applied at the times shown by arrows.

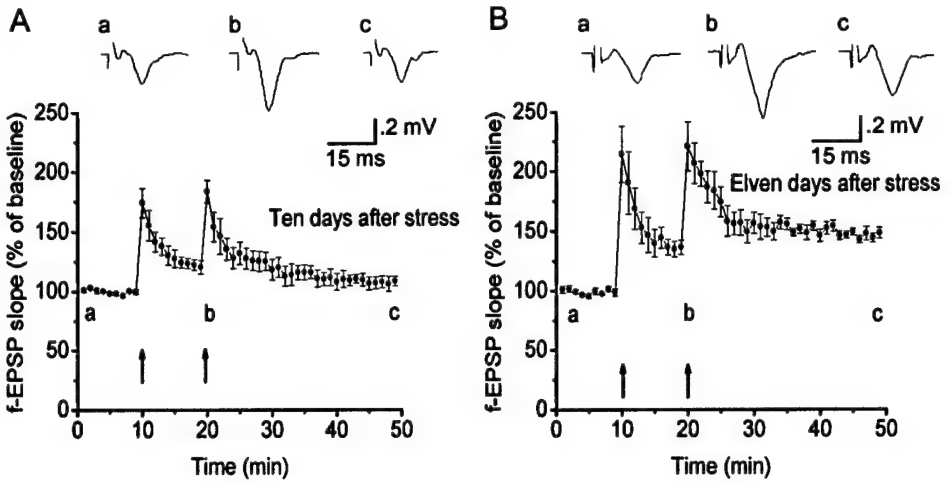


Figure 23, Three days stress impairs two TBS-induced LTP 10 days after the termination of stress paradigm(A). The two TBS-induced LTP resumed 11 days after the termination of stress (B).

Stress impairs two theta burst stimulations induced long-term potentiation in the Rat Basolateral Amygdala *in vitro*

We have examined the impact of single and repeat stress on theta burst stimulation induced enduring synaptic potentiation in the basolateral amygdala in vitro, and demonstrated that two theta burst stimulations (applied at 10 minute intervals) of the external capsule (EC) induced a persistent enhancement in the slopes of homosynaptic potentiation in the intercellular recorded single neuron and field potentials recorded in basolateral amygdala circuitry. Single theta burst stimulation induces a short-term synaptic potentiation lasting around 10 minutes after the termination of stimulation. The enhancement of two theta burst stimulation induced synaptic potentiation was maintained for more than 30 min following termination of the stimulus train. Two TBS-induced enduring synaptic facilitation was blocked by the N-methyl-D-aspartate (NMDA) receptor antagonist D-2-amino-5-phosphonovalerate (APV; 100 μM). The acute one day stress paradigm impairs the induction of two TBS-induced long term potentiation with no significant effect on a single TBS induced short-term potentiation. The impairment of two TBS induced LTP by one day stress lasted for three days and the two TBS induced LTP was resumed on the fourth day after the termination of stress. Repeat of the three day stress paradigm also impairs the induction of two TBS-induced long-term potentiation with no significant effect on a single TBS induced short-term potentiation. The impairment of two TBS induced LTP by three days stress lasted for ten days and the two TBS induced LTP resumed on the eleventh day after the termination of stress. (Figure 23). The slope of f-EPSPs 30 min after TBBS was 148.2±8.9% (n=4, Figure 23B) of the initial baseline values. The result suggests that the recovery of the ability to induce LTP with TBS is a time-dependent recovery from the stress.

The present findings provide insight into possible mechanisms underlying the impact of stress on emotional learning and memory. These results indicate that stressful stimuli have mild impact on the induction of short-term synaptic plasticity, but prolonged effects on the induction of long-term synaptic plasticity in the basolateral amygdala. The time course of resilience from such stressful stimuli is correlated with the intensity of the stressors.

Key Research Accomplishments

(1) We have successfully established and tested the inescapable tail-shock model of stress in rats and verified that long-lasting behavioral and physiological effects, known to be mediated by the amygdala, result from applying the inescapable tail-shock stress paradigm to the animals studied.

(2) We have characterized the role of kainate receptors containing the GluR5 subunit in synaptic plasticity, long term potentiation (LTP) and long term depression (LTD) in the basolateral amygdala.

(3) We have also established the modulatory role of the GluR5-containing kainate receptors in inhibitory neurotransmitter release in the basolateral amygdala of control and traumatically stressed rats.

(4) By using intracellular and field recording, as well as the patch clamp technique, we have characterized the role of α_1 adrenergic ($\alpha_{1\,A}$, $\alpha_{1\,B}$, and α_{1D}) receptors in synaptic transmission, calcium signaling and neuroplasticity of the basolateral amygdala of naïve and stressed rats.

(5) By using the patch clamp technique, we have characterized the role of α_2 adrenergic receptors in modulating inhibitory synaptic transmission in the basolateral amygdala of naïve rats.

(6) By using intracellular and field potential recordings, as well as the patch clamp technique, we have characterized the role of β adrenergic receptors in synaptic transmission, calcium signaling and neuroplasticity of the basolateral amygdala of naïve and stressed rats.

(7) We have characterized relevant aspects of the effects of the serotonergic neurotransmission in the basolateral amygdala of control rats and rats subjected to our inescapable tail-shock paradigm.

(8) We have demonstrated that stressful stimuli have mild impact on the induction of short-term synaptic plasticity but has prolong effects on the induction of long-term synaptic plasticity in the basolateral amygdala. The time course of resilience from such stressful stimuli is correlated with the intensity and duration of the stressors.

Reportable Outcomes

MANUSCRIPTS:

1. Braga, M., Aroniadou-Anderjaska, V. and **Li, H.** :The physiological role of kainate receptors in the amygdala. An invited review paper for ***Molecular Neurobiology*** (in press, 2004).

2. Braga, M. F., Aroniadou-Anderjaska, V., Manion, S.T., Hough, C.J., and **Li, H.:** Stress impairs α_{1A} adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala. ***Neuropsychopharmacology***. 29,45- 58, 2004

3. Chen, A., Hough, C.J. and **Li, H.**: 5-HT_2 Receptor activation facilitates synaptic plasticity via NMDA-mediated mechanism in the basolateral amygdala. ***Neuroscience.*** 119: 53-63, 2003

4. Braga, M. F., Aroniadou-Anderjaska, V., Xie, J, and **Li, H.** : Bidirectional modulation of GABA release by presynaptic glutamate 5 kaiante receptors in the basolateral amygdala. ***Journal of Neuroscience***. 23(1): 442-452, 2003.

5. Rogawski, M.A., Gryder, D., Castanada, D. Yonekawa, W. Banks, M.K., **Li, H.**:Kainate receptor mediated long-term plasticity, seizures and epileptogenesis in the amygdala. ***Annal of the New York Academy of Sciences***, 985:150-162, 2003

6. Post, R.M, Leverich, G.S., Weiss, S.R.B., Zhang, L.X., Xing, Xing, G.Q., **Li, H.** and Smith, M.: Psychosocial Stressors as predisposing factors to affective illness and PTSD: Potential neurobiological mechanisms and theoretical implications. ***Neurodevelopmental Mechanisms in Psychopathology***, Cambridge University Press, chapter 20, 491-525, 2003

7. Braga, M.F.M., Aroniadou-Anderjaska, V., Post, R.M. and **Li, H.**: Lamotrigine reduces spontaneous and evoked GABAA receptor-mediated synaptic transmission in the basolateral amygdala: Implications for its effects in seizure- and affective disorders ***Neuropharmacology***, 42:522-529, 2002.

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10. Rogawski, M., Hurzman, P., Yamaguchi, S. and **Li, H.**: Role of AMPA and GluR5 kainate receptors in the development and expression of amygdala kindling in the mouse. ***Neuropharmacology,*** 40(1): 28-35, 2001

LAY PUBLICATIONS

Li, H. : Understanding Cellular Mechanisms of Post-traumatic Stress Disorder: Studies of Synaptic Function in Amygdala. ***Washington Psychiatric society news*** pg.7, September-October, 2000

ABSTRACTS/PRESENTATIONS

Maria F. M. Braga, Viky Aroniandou-Anderjaska, Christopher J. Hough, Sean Manion and **He Li**, Stress impairs adrenoceptor-mediated noradrenergic facilitaiton of GABAergic transmission in the basolateral amygdala. Submitted to Society for Neuroscience Meeting, (2003).

Maria F. M. Braga, and **He Li**, Topiramate enhance GABAergic transmission and blocks GluR5 kainate receptor in basolateral amygdala interneurons. Submitted to Society for Neuroscience Meeting, (2003).

Sean Manion, Maria F. Braga and **He Li**, Effect of traumatic stress on noradrenergic-mediated modulation of neuronal excitability and neuroplasticity in the Amygdala. Submitted to Society for Neuroscience Meeting, (2003).

Maria F. Braga, Viky Aroniandou-Anderjaska, Christopher J. Hough, Sean Manion and **He Li**: Stress impairs α1A adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala. Uniformed Services University Research Day, p46, The President's Selected Poster Session (2003).

Maria F. Braga, Viky Aroniandou-Anderjaska, Christopher J. Hough, Sean Manion and **He Li**: Stress impairs α1A adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala. Poster presentation in the Conference of Roots of Mental Illness in Children, Annals of the New York Academy of Sciences (2003).

Aiqin Chen and **He Li**. Roles of Protein Kinase C and Protein Tyrosine Kinase in Theta Burst Stimulation-Induced Synaptic Potentiation in the Basolateral Amygdala. Uniformed Services University Research Day, p41, Neuroscience Session (2003).

Sean Manion, Maria F. Braga and **He Li**, Effect of traumatic stress on noradrenergic-mediated modulation of neuronal excitability and neuroplasticity in the Amygdala. Uniformed Services University Research Day, (2003).

Aiqin Chen, Michael A. Rogawski, Robert M. Post and **He Li** : Biphasic effects of GluR5 kainate receptor agonist on synaptic transmission in basolateral amygdala *in vitro.* (The Amygdala in Brain Function: Basic and Clinical Approaches A New York Academy of Sciences Conference, p35, 2002)

Michael A Rogauski, Divina Gryder, Dora Castanada, Wayne Yonekawa and **He Li** : Kainate receptor mediated long-term plasticity, seizures and epileptogenesis in the amygdala. (The Amygdala in Brain Function: Basic and Clinical Approaches A New York Academy of Sciences Conference, p11, 2002).

LONGKUN ZHU, AIQIN CHEN AND **HE LI**: ENHANCEMENT OF NMDA SYNAPTIC RESPONSE BY

GROUP I METABOTROPIC GLUTAMATE RECEPTOR AGONIST tADA IN BASOLATERAL AMYGDALA. SOCIETY FOR NEUROSCIENCE MEETING, (2002).

PRESENTATIONS:

(1) He Li: GluR5 kainate receptor and neuroplasticity in the amygdala. **National Institute of aging**, November, 2001.

(2) He Li: GluR5 kainate receptor mediated synaptic functions in the amygdala. **National Institute of Mental Health**, April, 2002.

(3) Maria F. M. Braga: Chronic Stress Causes Impairment of α_1-Adrenoceptor-mediated Modulation of GABAergic Synaptic Transmission in the Basolateral Amygdala. **Graduate Student Colloquium and Research Day**, USUHS, 2002.

(4) Maria F. M. Braga: Bidirectional Modulation of GABA Release by Presynaptic GluR5 Kainate Receptors in the Basolateral Amygdala. **USUHS Post-Doctoral Student Association Seminar Series**, 2003.

Conclusion

Our results indicate that we have successfully established and tested the inescapable tail-shock model of stress in rats and verified that long-lasting behavioral and physiological effects, known to be mediated by the amygdala, result from applying the inescapable tail-shock stress paradigm to the animals studied. We have characterized the role of kainate receptors containing the GluR5 subunit in synaptic plasticity, long term potentiation (LTP) and long term depression (LTD) in the basolateral amygdala. We have also established the modulatory role of the GluR5-containing kainate receptors in inhibitory neurotransmitter release in the basolateral amygdala of control and traumatically stressed rats. By using intracellular and field recording, as well as the patch clamp technique, we have characterized the role of α_1 and α_2 adrenoceptors in synaptic transmission, calcium signaling and neuroplasticity of the basolateral amygdala of naïve and stressed rats. By using intracellular and field potential recordings, as well as the patch clamp technique, we have characterized the role of β adrenergic receptors in synaptic transmission, calcium signaling and neuroplasticity of the basolateral amygdala of naïve and stressed rats. Finally, We have characterized the facilitating effects of the serotonin type II receptor on the induction of long-term potentiation in the basolateral amygdala of control rats and rats subjected to our inescapable tail-shock paradigm. We have demonstrated that stressful stimuli has mild impact on the induction short-term synaptic plasticity but has prolong effect on the induction of long-term synaptic plasticity in the basolateral amygdala. The time course of resilience from such stressful stimuli is correlated with the intensity and duration of the stressors.

The identification of these multiple cellular mechanisms affected by stress in the amygdala will provide important detailed information for developing pharmacological interventions to regulate circuit excitability in the amygdala and to ameliorate amygdala related mental disorders such as fear, anxiety, depression and PTSD.

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APPENDIX 1

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SEROTONIN TYPE II RECEPTOR ACTIVATION FACILITATES SYNAPTIC PLASTICITY VIA *N*-METHYL-D-ASPARTATE-MEDIATED MECHANISM IN THE RAT BASOLATERAL AMYGDALA

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Abstract—The modulation of synaptic plasticity by serotonin type II (5-hydroxytryptamine type II (5-HT_2))-receptor stimulation was explored using intracellular, field potential and Fura-2 fluorescence image recordings in a rat amygdala slice preparation. Bath application of $5HT_2$ receptor agonist 1-(2,5)-dimethoxy-4-iodophen-2-aminopropane (DOI) transformed θ-burst-stimulated (TBS) synaptic plasticity from short-term potentiation to long-term potentiation. DOI enhanced *N*-methyl-D-aspartate (NMDA) receptor-mediated potentials and calcium influx without affecting the resting membrane potential or input resistance of the neurons. In contrast, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate receptor-mediated excitatory synaptic responses were unaffected by DOI. The facilitating effects of DOI were blocked by the 5-HT_2 receptor antagonist, ketanserin, and by the 5-HT_{2C}-receptor selective antagonist, RS102221. These results indicate that 5-HT_2-receptor activation enhances NMDA receptor-mediated synaptic function in the basolateral amygdala (BLA). Published by Elsevier Science Ltd on behalf of IBRO.

Key words: DOI, serotonin, synaptic plasticity, LTP, calcium, fear.

The amygdala complex is well known for its involvement in mood and emotion (Davis, 1992; Ledoux, 1995), and may participate in the pathogenesis of schizophrenia (Bogerts et al., 1985), depression (Drevets et al., 1992), epilepsy (Boucsein et al., 2001; Pitkanen et al., 1998) and posttraumatic stress disorder (PTSD) (Liberzon et al., 1999; Post et al., 1998; Rauch et al., 2000). In these illnesses, the importance of serotonergic neurotransmission is universally acknowledged. Enhanced serotonin release during periods of anxiety and stress has been observed in the amygdala complex (Fernandes et al., 1994). The amygdala expresses high levels of serotonin type II (5-hydroxytryptamine type II (5-HT_2)) receptor protein and mRNAs (Morilak et al., 1994; Pompeiano et al., 1994; Wright et al., 1995). Activation of 5-HT_2 receptors in the amygdala facilitates the development of amygdaloid kindling (Wada et al., 1997) and has a profound effect upon anxiety and mood (Hrdina et al., 1993; Kshama et al., 1990; Tokuyama et al., 1993).

Excitatory neurotransmission in the basolateral amygdala (BLA) is mediated by *N*-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate receptors (Li and Rogawski, 1998; Rainnie et al., 1991). This excitatory neurotransmission exhibits NMDA receptor-dependent and -independent long-term synaptic plasticity (Gean et al., 1993; Li et al., 1998, 2001; Maren, 1999). Synaptic plasticity can be negatively modulated in the visual cortex by 5-HT_2-receptor stimulation (Edagawa et al., 2000). The effect of 5-HT_2-receptor modulation of synaptic plasticity of amygdala circuitry, however, remains to be elucidated. This synaptic plasticity may underlie the learning of traumatic memories that characterize fear conditioning, anxiety disorders and posttraumatic stress disorder.

In the study reported here, we characterize the excitatory effect of 5-HT_2-receptor stimulation in the basolateral amygdala, using electrophysiological and calcium imaging techniques. The results demonstrate that 5-HT_2-receptor activation facilitates NMDA receptor-dependent synaptic potentiation in the basolateral amygdala (BLA) by enhancing NMDA receptor-mediated calcium influx.

EXPERIMENTAL PROCEDURES

Amygdala slice preparation

Male Sprague–Dawley rats weighing 75–150 g (4–6 weeks) were used. The rats were decapitated, the brains rapidly removed, and transverse slices (500 μm thickness for intracellular recording and field potential recording, 350 μm thickness for calcium imaging) of the amygdala were cut from tissue blocks with a Vibratome (Technical Products International, St. Louis, MO, USA). The slices were preincubated in oxygenated artificial cerebrospinal fluid (ACSF) continuously bubbled at room temperature (25 °C) with 95% O_2/5% CO_2 for at least 1 h before use. The ACSF contained (in mM) 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, and 11 glucose, and was bubbled with 95% O_2/5% CO_2 to maintain a pH of 7.4. Experiments carried out in amygdala

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate; APV, (±)-2-amino-5-phosphonovaleric acid; Bic, bicuculline methochloride; BLA, basolateral amygdala; DOB, (−)-1-2,5-dimethoxy-4-bromophenol-2-aminopropane; DOI, (±)-1-(2,5-dimethoxy-4-iodophen)-2-aminopropane hydrochloride; EC, external capsule; EPSP, excitatory postsynaptic potentials; Fura-2 AM, fura-2 acetoxymethyl ester; 5-HT_2, 5-hydroxytryptamine type II; LTP, long-term potentiation; LY293558, {(3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazol-5-yl)ethyl]-decahydroisoquinoline-3-carboxylic acid); NMDA, *N*-methyl-D-aspartate; PKC, protein kinase C; PPF, paired-pulse facilitation; PTSD, posttraumatic stress disorder; RS102221, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride; SCH50911, (2s)-(+)-5,5-dimethyl-2-morpholineacetic acid; TBS, θ-burst stimulation; WCP, Whole Cell Electrophysiology Program.

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slices were perfused with ACSF containing normal physiological concentration of magnesium unless otherwise stated. All animals were cared for and used experimentally to reduce suffering in accordance with the *Guide to the Care and Use of Laboratory Animals* and all experiments were approved by the Uniformed Services University Animal Care Committee. Amygdala slices from each animal were shared within the laboratory whenever possible in order to minimize the number of animals used.

Intracellular and field-potential recordings

Slices were transferred to an interface chamber that was continually superfused with ACSF at a rate of 1–2 ml/min. Microelectrodes were pulled from microfiber-filled borosilicate capillaries (OD 1.0 mm, ID 0.58 mm for intracellular recording; OD 1.0 mm, ID 0.78 mm for extracellular recording) using a Flaming-Brown horizontal micropipette puller (Sutter Instruments, Novato, CA, USA). The resistance of the intracellular microelectrodes filled with 3 M KCl ranged from 80 to 130 MΩ, while extracellular recording electrodes were filled with 3 M NaCl and the resistance was 2–5 MΩ. The microelectrode tips were visually positioned in the basolateral region of the amygdala (between the external capsule [EC] and the bed nucleus of the stria terminalis) using a dissecting microscope. Intracellular impalements were made in a blind fashion. Intracellular recordings were terminated if the resting membrane potential was more positive than −55 mV or if the action potential height was less than 70 mV. Intracellular potential was amplified with an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA, USA; low-pass filter, 3 kHz). Field potential was amplified with a differential amplifier (Warner Instrument Corporation, Hamden, CT, USA). The output was digitized with a Digidata 1200 interface (Axon Instruments, Foster City, CA, USA). On- and off-line data acquisition and analysis was carried out using Whole Cell Electrophysiology Program (WCP) version 1.7b (John Dempster, University of Strathclyde, Glasgow, UK). Intracellular recordings were made from pyramidal neurons. Baseline intracellular and field recordings were established for 20 min before application of θ-burst stimulation (TBS). Excitatory postsynaptic potentials (EPSP) slopes and amplitudes were normalized to this averaged baseline value (100%). The maximum rate of EPSP within 10%-90% rising phase was measured as the slope of EPSP using the WCP software. NMDA receptor-mediated synaptic transmission was isolated by bath perfusion of 10 μM bicuculline methochloride (Bic), 10 μM (2s)-(+)-5,5-dimethyl-2-morpholineacetic acid (SCH50911) and 20 μM LY293558 to block $GABA_A$, $GABA_B$ and AMPA/kainate synaptic components respectively.

Stimulation

Synaptic responses were evoked with sharpened tungsten bipolar stimulating electrodes (World Precision Instruments, Sarasota, FL, USA) placed in the EC. The stimulating electrode was approximately 2 mm from the recording site. Stimuli were delivered using a photoelectric stimulus isolation unit having a constant current output (ISO-Flex; Stimulus Isolation Unit, Jerusalem, Israel). The stimulus intensity was adjusted to produce a synaptic response of less than 50% of the maximum amplitude obtainable without triggering an action potential response. Peak response amplitudes were measured with respect to the resting membrane potential. Single 0.1 ms monophasic square pulses were applied continuously throughout the experiment at 0.1 Hz. To induce synaptic plasticity, TBS (a brief, high-frequency pulse train of five pulses at 100 Hz given at the θ rhythm, 5 Hz, for 4 s) was applied at the same intensity through the same electrode as used for the test stimulation. The TBS protocol mimics the typical firing mode of pyramidal cells during learning (Otto et al., 1991).

Calcium measurement

Amygdala slices were incubated with ACSF containing 15 μM fura-2 acetoxymethyl ester (Fura-2 AM) and 0.02% Pluronic F127 at 37 °C for 30 min and then rinsed in ACSF at room temperature for an additional 15–30 min to remove unincorporated Fura-2 AM. The slices were transferred to a chamber mounted on an upright Zeiss microscope (Carl Zeiss Inc., Thornwood, NY, USA), submerged and superfused with ACSF at 2 ml/min at room temperature. The microscope was coupled to a DeltaRam monochromator (PTI, Monmouth Junction, NJ, USA) and excitation wavelengths were set to 340 nm and 380 nm. Emitted fluorescence images at 510 nm or higher were captured at a rate of 2 Hz through a 63× Zeiss water immersion objective (N.A. 0.95) with a digital CCD camera (ORCA 100, Hamamatsu, Tokyo, Japan) and collected using OpenLab imaging software from Improvision (Lexington, MA, USA). The ratio of emission fluorescence intensity of neuronal somata at 340 nm and 380 nm was calculated with the Improvision software. Peak heights were defined as the difference between the maximum fluorescence ratio value of the smoothed peak and the baseline averaged over 10 s immediately preceding the peak.

Drug application

Chemicals and drugs including (±)-2-amino-5-phosphonovaleric acid (APV), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI), ketanserin, NMDA and glycine were from Sigma (St. Louis, MO, USA). {(3*S*,4a*R*,6*R*,8a*R*)-6-[2-(1(2)*H*-tetrazol-5-yl)ethyl]-decahydroisoquinoline-3-carboxylic acid} (LY293558) was a generous gift of Lilly Research Laboratories (Indianapolis, IN, USA). Drugs including (−)-Bic, SCH50911, and the selective 5-HT_{2C} receptor antagonist 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro-[4.5]decane-2,4-dione hydrochloride, (RS102221) were from Tocris (Ellisville, MO, USA). Fura-2 acetoxymethyl ester (Fura-2 AM) and Pluronic F-127 were from Molecular Probes (Eugene, OR, USA).

Statistical analysis

Data are expressed as mean±S.E.M. *t*-Tests were performed. Statistical comparisons were made with the paired *t*-test for paired data within the group. Two-sample unequal variance *t*-test was used for the data between the groups.

RESULTS

DOI facilitates TBS-induced synaptic plasticity

We first addressed whether 5-HT_2-receptor activation by DOI could affect TBS-induced synaptic plasticity in the BLA. Both intracellular and field synaptic potentials were potentiated immediately following TBS and gradually returned to baseline levels within approximately 10 min (Fig. 1). The slope of EPSPs 30 min after TBS was 96.8±2.2% (n=5, P>0.05) of the initial baseline values for intracellular and 119.5±5.1% (n=5, P>0.05) for field potentials.

Bath application of 100 μM DOI for 15–20 min had no observable effect on the amplitude of basal synaptic responses. The peak amplitude of intracellular EPSPs and field potentials was 99.7±2.2% (P>0.05, n=6) and 94.9±5.3% (P>0.05, n=6) of the baseline values after drug administration. However, TBS-induced synaptic potentiation was significantly facilitated and remained at potentiated levels for more than 30 min in all the slices treated with DOI. As illustrated in Fig. 2A and B (black

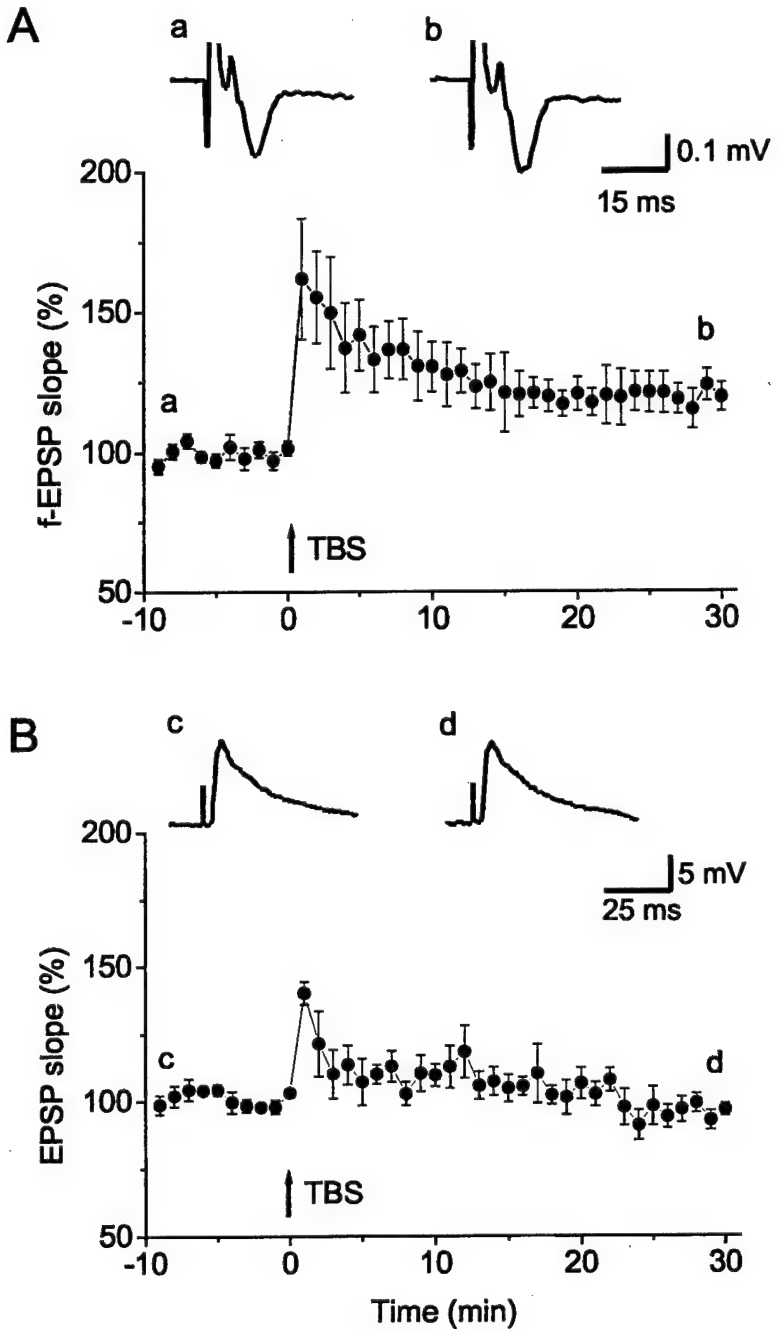

Fig. 1. Single TBS induces short-term synaptic potentiation of intracellular EPSPs and field potentials (fEPSPs) in the basolateral amygdala. (A, B) Summary graphs of fEPSP (A, n=5) and EPSP (B, n=5) slopes were plotted as a function of time, respectively. The values are expressed as a percentage of the mean of 60 responses at 0.1 Hz before the application of TBS. Each point represents the mean±S.E.M. TBS was applied at time 0 (as shown by arrow). Insets show typical traces of EPSPs or fEPSPs at the times indicated. Traces are the averages of six consecutive responses to the stimulation at 0.1 Hz.

circles), the potentiated slopes of field potentials and EPSPs remained at 164.7±11.8% (n=7, P<0.01) and 166.6±31.0% (n=5, P<0.05), respectively, of the baseline values 30 min after the onset of TBS.

To examine whether the effect of DOI on facilitating TBS-induced synaptic potentiation is dependent upon NMDA-receptor activation, the NMDA-receptor antagonist APV (100 μM) was also applied with the DOI. Under this condition, TBS was no longer able to induce long-lasting synaptic facilitation, and the slope of field potentials was 115.4±5.1% (P>0.05, n=4, Fig. 2A) of the baseline values 30 min after the TBS. These results indicate that activation of NMDA receptors by TBS is required for DOI to facilitate synaptic plasticity in the BLA. In addition, these results suggest that DOI may enhance NMDA channel function and therefore facilitate

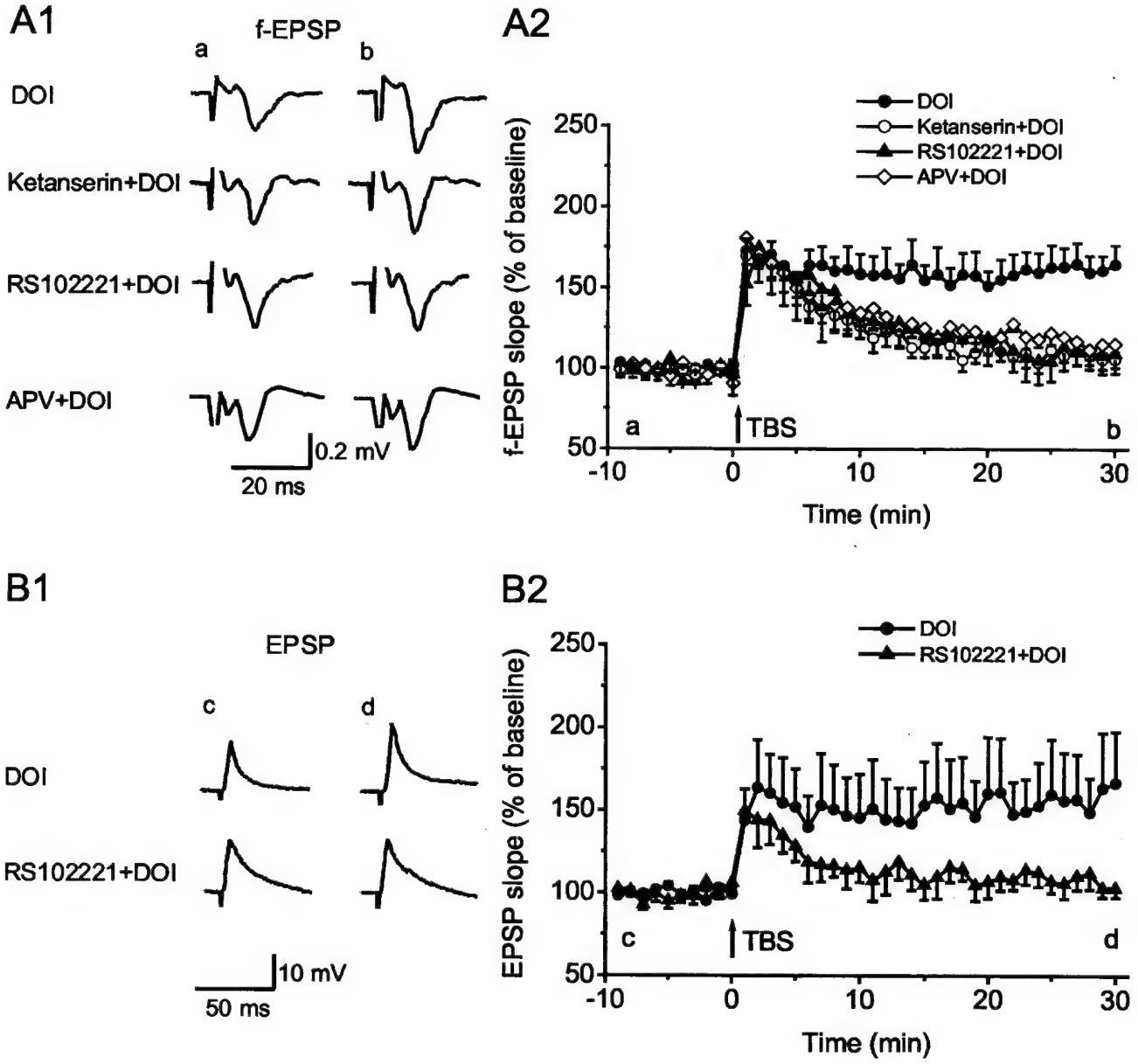

Fig. 2. Effects of 5-HT_2 receptor agonist, antagonist and NMDA receptor antagonist on TBS-induced synaptic plasticity. A1, Sample records of evoked fEPSPs before (a) and 30 min after (b) TBS in the presence of 100 μM DOI alone and in the presence of ketanserin (20 μM), RS102221 (5 μM) or APV (100 μM). Traces are the averages of six consecutive responses to EC stimulation at 0.1 Hz. (A2) Summary fEPSP slopes were plotted as a function of time in the presence of 100 μM DOI alone (black circles, n=5), and 100 μM DOI in the presence of ketanserin (20 μM, white circles, n=5), RS102221 (5 μM, black triangles, n=4), or APV (100 μM, white diamond, n=4). (B1) Sample records of evoked EPSP before (c) and 30 min after (d) TBS in the presence of 100 μM DOI alone or in the presence of RS102221 (5 μM). Traces are the averages of six consecutive responses to EC stimulation at 0.1 Hz. (B2) Summary EPSP slopes were plotted as a function of time in the presence of 100 μM DOI alone (black circles, n=7) or accompanied by RS102221 (5 μM, black triangles, n=3). TBS was applied at time 0 (indicated by arrow). The slopes of fEPSPs (A2) and EPSPs (B2) are expressed as a percentage of the averaged basal responses before TBS.

the TBS-induced synaptic plasticity in amygdala circuitry.

The facilitation effect of DOI is mediated by activation of 5-HT_2 receptor

To address the question of what subtype of the 5-HT_2 receptor may be responsible for the facilitation effect of DOI, the selective 5-HT_2 antagonist ketanserin and the 5-HT_{2C} antagonist RS102221were applied (Bonhaus et al., 1997). The highly selective 5-HT_{2C}-receptor antagonist RS102221 was used because 5-HT_{2C} receptors are present at very high levels in the amygdala (Pompeiano et al., 1994). Application of ketanserin (20 μM) or RS102221 (5 μM) had no significant effect on basal synaptic responses in the BLA. However, after pretreatment of the slices with ketanserin or RS102221 for 15

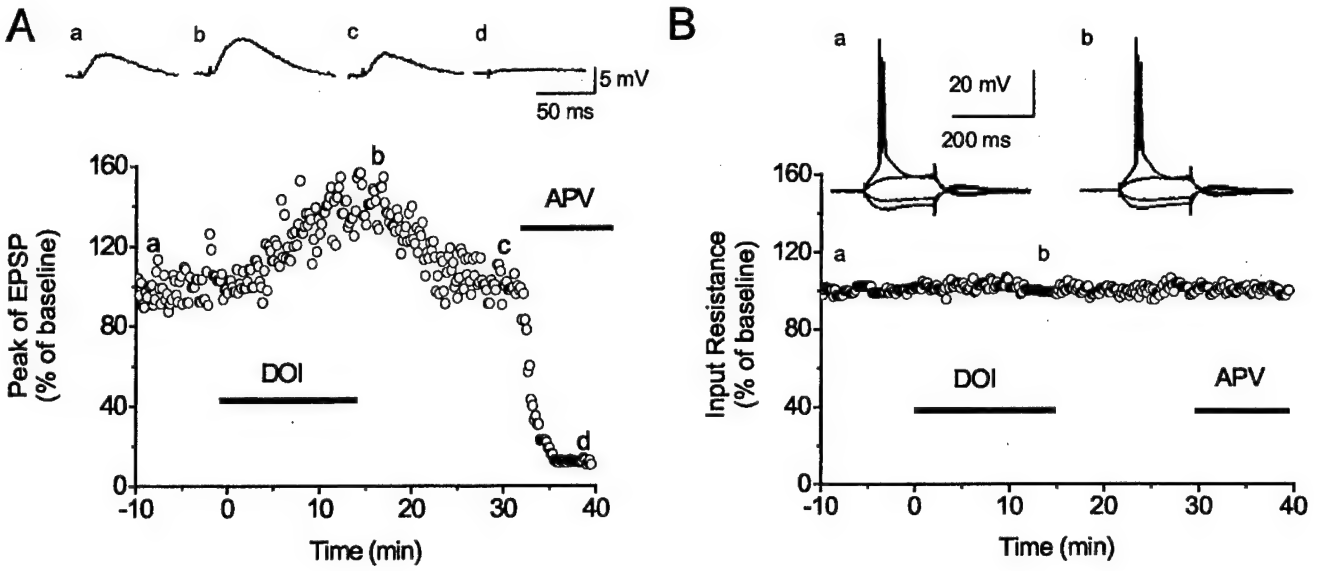

Fig. 3. The effect of DOI on NMDA receptor-mediated EPSPs. (A) NMDA receptor-mediated EPSPs after administrating DOI (100 μM) for 15 min. The sample traces were taken at the times indicated by the letters a, b and c. These traces are the averages of six consecutive responses. NMDA receptor-mediated EPSPs after bath application of DOI (100 μM) and after perfusion with APV (100 μM) are presented. Bars denote the delivery periods of DOI (100 μM) and APV (100 μM). (B) The input resistances (monitored by a 100 pA hyperpolarizing current pulse passed through the recording electrode) in the presence and absence of DOI (100 μM). Inserted traces are the voltage responses of the neuron to injected current (±100 pA, ±200 pA) at the times before (Ba) and during (Bb) the application of DOI (100 μM). Input resistance is expressed as a percentage of averaged responses before DOI. Bar denotes the period of application of DOI (100 μM).

min, DOI was no longer able to facilitate TBS-induced synaptic plasticity. As illustrated in Fig. 2A (white circles), the field EPSPs were 105.7±8.5% (n=5) of the baseline values 30 min after the TBS in the presence of ketanserin and DOI. In the presence of RS102221 and DOI, the field EPSPs and intracellular EPSPs 30 min after the TBS were 106.4±8.8% (n=3) and 102.8±5.7% (n=4) of the basal values, respectively (Fig. 2A, B, black triangles).

DOI specifically enhances postsynaptic NMDA receptor functions

To determine whether the facilitating effect of DOI on TBS-induced synaptic plasticity is related to an enhancement of NMDA-receptor function, the effect of DOI on NMDA receptor-mediated synaptic transmission in normal ACSF was examined in the BLA. NMDA receptor-mediated synaptic transmission was isolated by bath perfusion of 10 μM Bic, 10 μM SCH50911 and 20 μM LY293558 to block $GABA_A$, $GABA_B$ and AMPA/kainate synaptic components respectively. The resting membrane potential and input resistance remained unaffected by the application of 100 μM DOI (Fig. 3B). The mean resting potential of neurons before and 15 min after the administration of DOI was −69±1 and −68±1 mV (n=12), respectively. In these experiments, the mean input resistances determined from the voltage response to 100 pA hyperpolarizing current pulses injected through the recording electrode were 58.9±2.5 and 59.1±2.2 MΩ (n=12), respectively before and 15 min after administration of DOI. In most experiments, voltage responses and action-potential firing patterns evoked by depolarizing current steps were also monitored before and after the application of DOI. As illustrated in Fig. 3B, the action potential firing patterns and the voltage changes obtained by injecting −100 pA, 150 ms current before, during and after the application of DOI were not altered in the BLA neurons, indicating that DOI did not significantly affect the electrophysiological proprieties of the membrane. However, bath application of 100 μM DOI for 15 min enhanced the peak amplitude of NMDA receptor-mediated synaptic transmission evoked by external capsule (EC) stimulation in intracellular recording in the BLA (Fig. 3A, Fig. 4A). The peak amplitude of NMDA receptor-mediated EPSPs enhanced by DOI was 144.1±6.8% of the baseline (P<0.01, n=12). The effect of DOI was evident 3–5 min following its application and was sustained for up to 30 min (n=3, 140.5±7.5% of intracellular recorded potentials). NMDA receptor-mediated EPSPs gradually returned to their initial levels after a 5–10 min washout with ACSF. The fact that the synaptic component enhanced by DOI was mediated primarily by NMDA receptors was confirmed at the end of these experiments by the application of NMDA receptor antagonist APV (100 μM) as illustrated in Fig. 3A. In addition, the facilitating effect of DOI on NMDA receptor-mediated EPSPs was prevented by the presence of the 5-HT_2 receptor antagonist ketanserin or the 5-HT_{2C} receptor antagonist RS102221, indicating that the DOI-induced effect appears to be mediated by 5-HT_2 subtypes (Fig. 4A).

For comparison, we tested the effect of DOI on AMPA/kainate receptor-mediated synaptic responses. AMPA/kainate receptor-mediated synaptic transmission was isolated using 10 μM Bic, 5 μM SCH50911 and 100 μM APV in the

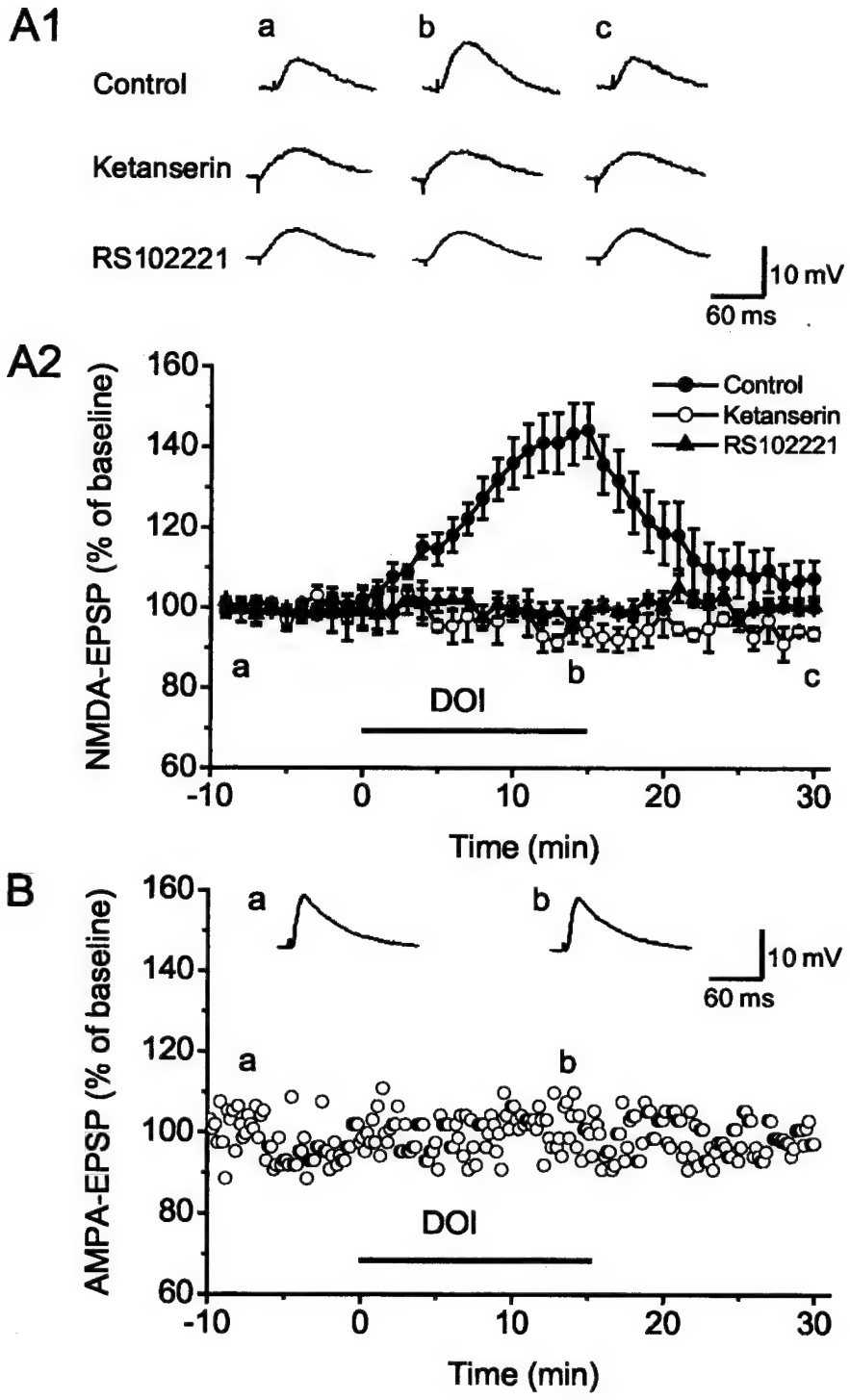

Fig. 4. Effects of DOI (100 μM) on NMDA or AMPA receptor-mediated EPSPs. (A1) NMDA receptor-mediated EPSPs before and after application of 100 μM DOI are shown. The effects of DOI on NMDA EPSPs are also shown in the presence of ketanserin (20 μM) or RS102221 (5 μM). (A2) The amplitudes of NMDA EPSPs are plotted as a function of time. For each experiment, synaptic potential amplitude is expressed as a percentage of averaged responses before DOI administration. The amplitude of NMDA EPSPs before and after addition of 100 μM DOI alone (black circles, $n=12$) or accompanied by ketanserin (white circles, $n=3$) or RS102221 (black triangles, $n=5$). (B) The effect of DOI (100 μM, 15 min) on AMPA/kainate receptor-mediated EPSPs in a representative experiment. The sample traces were taken before (a) and during (b) the application of DOI. Bar denotes the period of application of DOI (100 μM).

perfusion medium. In contrast to its enhancement of NMDA receptor-mediated synaptic responses, DOI failed to increase AMPA/kainate receptor-mediated synaptic transmission (Fig. 4B). The peak amplitude of AMPA/kai-

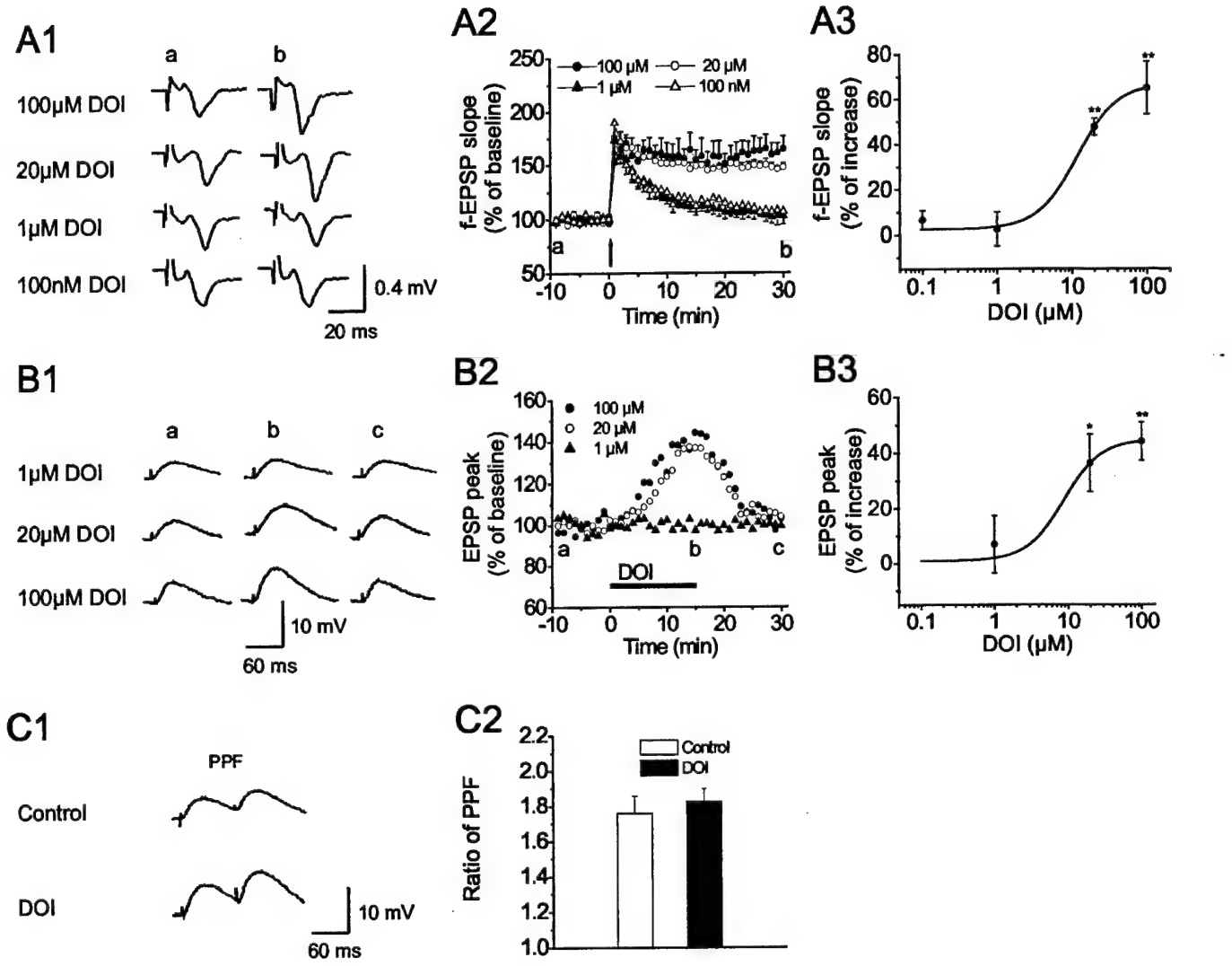

Fig. 5. Effects of different concentrations of DOI on TBS-induced plasticity, NMDA receptor-mediated EPSPs and PPF. (A) TBS induces synaptic potentiation in the presence of 0.1 μM, 1 μM, 20 μM and 100 μM DOI. (A1) Sample traces of fEPSPs are presented before (a) and 30 min after (b) TBS. Each trace is the averages of six consecutive responses to EC stimulation at 0.1 HZ. (A2) Summary results of fEPSP slope in the presence of DOI at the concentrations of 0.1 μM (white triangles, n=7), 1 μM (black triangles, n=4), 20 μM (white circle, n=5) and 100 μM (black circles, n=5) are plotted as a function of time. (A3) The percentage increase of fEPSP slope at the time of 30 min after (b) TBS is plotted against the concentrations of DOI on a logarithmic scale (0.1 μM, n=7; 1 μM, n=4; 20 μM, n=5, ** P<0.01 and 100 μM, n=7, ** P<0.01). (B) Effects of DOI on the amplitudes of NMDA EPSPs at the concentrations of 1 μM, 20 μM and 100 μM. Sample traces (B1) are the six consecutive averaged NMDA EPSP at the times indicated in B2. (B3) The peak amplitudes of NMDA EPSP are plotted against the concentrations of DOI on a logarithmic scale (1 μM, n=7; 20 μM, n=5, * P<0.05 and 100 μM, n=12, **P<0.01). (C1) Typical traces of NMDA EPSP evoked by paired-pulse facilitation (PPF) protocol before and after the administration of DOI (100 μM) are presented. (B2) Histograms represent the cumulative results from 11 experiments.

nate EPSPs evoked by EC stimulation was 95.7±4.7% (P>0.1, n=4) of the baseline values 15 min after the addition of 100 μM DOI.

The effect of DOI on TBS-induced long-term potentiation (LTP) and NMDA receptor-mediated synaptic transmission were examined using different concentrations of DOI. As indicated in Fig. 5A and 5B, DOI significantly facilitated TBS-induced LTP at concentration of 20 μM and 100 μM but not at 1 μM or 100 nM. The estimated EC50 is around 10 μM (Fig. 5A1–A3). In parallel, NMDA receptor-mediated EPSPs were enhanced by adminstration of DOI at concentrations of 20 μM as indicated in Fig. 5B1–B3 (136.4±10.3%, P<0.01, n=5) and 100 μM (144.1±6.8%, P<0.01, n=12) but not at 1 μM (107±10.5%, P>0.05, n=7).

A paired-pulse facilitation (PPF) stimulating protocol, in which two pulses are separated by a 60 ms interval, was used in an attempt to assess whether a presynaptic mechanism could explain the action of DOI on NMDA receptor-mediated EPSPs. Typical traces of NMDA receptor-mediated EPSPs elicited by a PPF-stimulating protocol are presented in Fig. 5C1. Bath application of DOI (100 μM) consistently led to an increase in the amplitude of both NMDA receptor-mediated EPSPs obtained. However, the ratio of these two pulse-evoked responses remained unchanged, suggesting a postsyn-

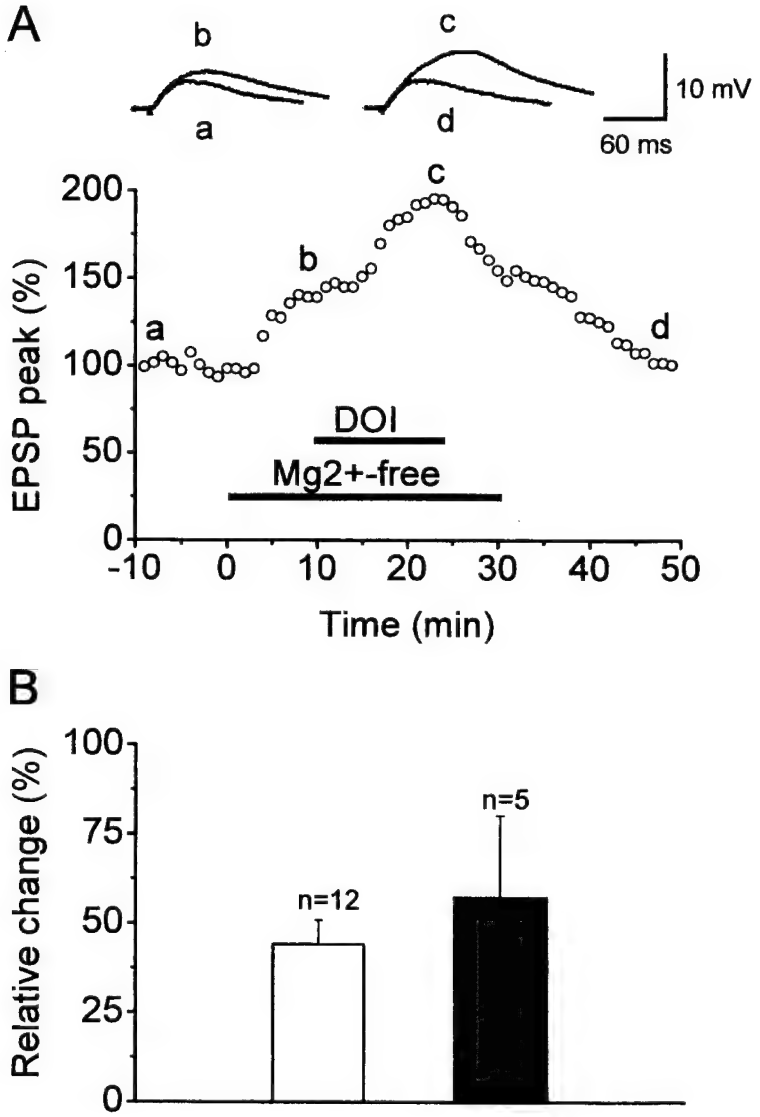

Fig. 6. Effect of DOI on NMDA receptor-mediated EPSPs in Mg^{2+}-free ACSF. (A) NMDA receptor-mediated EPSPs in Mg^{2+}-free ACSF in the presence DOI (100 μM). The sample traces were taken at the time indicated. Bars denoted the periods of delivery of Mg^{2+}-free ACSF and DOI (100 μM). (B) Histogram illustrates the cumulative results obtained with DOI (100 μM) in ACSF with normal Mg^{2+} (1.2 mM) and without Mg^{2+}.

aptic mechanism for DOI action. The results of 11 experiments are summarized in Fig. 5C2; the ratio value of PPF was 1.76±0.09 in control and 1.82±0.07 in the presence of DOI ($P>0.05$).

NMDA receptor-mediated synaptic potentials can also be enhanced by modulating the magnesium binding site of the NMDA receptor. To examine whether DOI is able to enhance NMDA-mediated potentials in the absence of magnesium, experiments were also carried out in magnesium-free medium (Fig. 6). NMDA receptor-mediated EPSPs evoked by EC stimulation were significantly enhanced to 130.9±9% of baseline ($P<0.01$, $n=5$) in Mg^{2+}-free ACSF (Arvanov et al., 1999). Under this condition, DOI (100 μM) was able to increase the NMDA receptor-mediated EPSPs by an additional 57.1±23.1% ($P<0.05$, $n=6$). The percent augmentation was equivalent to that obtained in the Mg^{2+} containing ACSF ($P>0.05$, $n=6$). These results suggest that 5-HT_2-receptor activation is able to modulate NMDA receptor functions independent of Mg^{2+} concentration in the medium.

Because the increase of intracellular calcium is considered a key event in the induction of LTP, we measured calcium influx elicited by NMDA-receptor stimulation of BLA neurons using Fura-2 fluorescence imaging in rat brain slices to examine whether DOI facilitation of NMDA-receptor function resulted in an enhancement of calcium entry. Bath perfusion included 10 μM Bic, 10 μM SCH50911 and 20 μM LY293558 to block GABA and AMPA/kainate receptors respectively. Calcium transients were elicited by a 60 s rapid perfusion of NMDA plus glycine (50 μM and 10 μM respectively). A second, identical application of NMDA with glycine, 120 s after

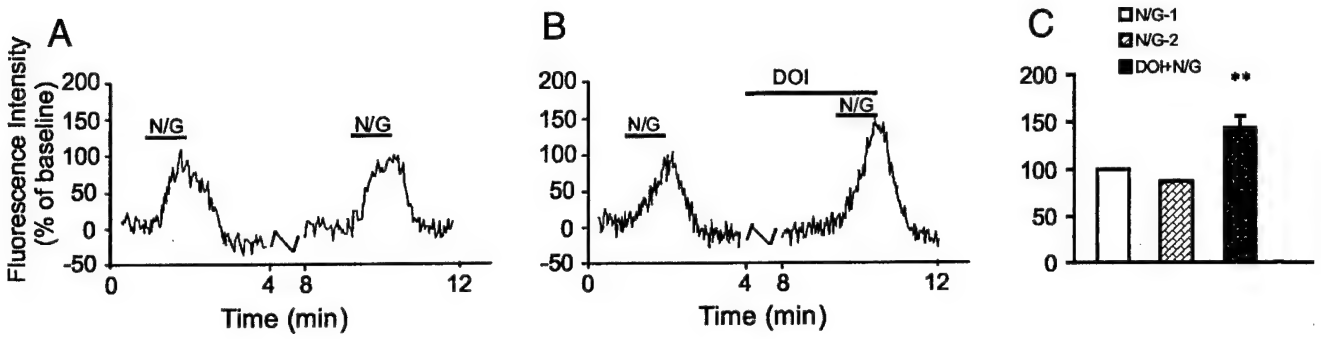

Fig. 7. The facilitating effect of DOI on NMDA-induced $[Ca^{2+}]_i$ response. (A) BLA neuron calcium responses to two brief (60 s) applications of NMDA plus glycine (50 μM and 10 μM, respectively) in the absence of DOI pretreatment. (B) Calcium responses obtained as in A before and after, respectively, a 5 min pretreatment with 100 μM DOI. (C) The cumulative results of $[Ca^{2+}]_i$ responses in the presence and absence of 100 μM DOI (** $P<0.01$, $n=19$).

the first, consistently induced calcium transients similar in amplitude to the first (87.3±1.7% of the first response, $n=4$, $P>0.05$). In this way, we compared NMDA-induced intracellular calcium responses first in the absence of and then in the presence of 100 μM DOI. The NMDA plus glycine-evoked calcium responses from selected neuronal soma in the BLA are illustrated by sample traces in Fig. 7A, B; cumulative data are summarized in Fig. 7C. After recovery from the first NMDA-induced calcium response, DOI (100 μM) was applied for 5 min before giving a second NMDA plus glycine application. Although no detectable calcium response was observed during the application of 100 μM DOI, the second NMDA with glycine-induced calcium response was enhanced as much as 143.0±13.4% of the first calcium response ($n=19$, $P<0.01$).

DISCUSSION

Activation 5-HT_2 receptor facilitates synaptic plasticity

Pharmacological blockade of 5-HT_2 receptors has a significant influence on mood and such amygdala-related physiological or pathological conditions as fear conditioning, anxiety and PTSD (Hertzberg et al., 1998; Huang and Kandel, 1998). 5-HT_2-receptor activation potentiates NMDA receptor-mediated currents (Blank et al., 1996), NMDA-induced depolarization of neocortical neurons (Rahman and Neuman, 1993) and amygdala kindling, a process that requires NMDA receptor activation (Wada et al., 1997). NMDA receptor-dependent and NMDA receptor-independent LTP can be elicited in the amygdala by different stimulating patterns (Chapman et al., 1990; Huang and Kandel, 1998; Li et al., 1998, 2001; Post et al., 1998). In general, a single, high-frequency stimulation induces short-term facilitation of synaptic transmission lasting about 10 min (Li et al., 1998). Long-lasting enhancement of synaptic efficacy requires two or more high-frequency trains of stimulation (Aroniadou-Anderjaska et al., 2001; Huang and Kandel, 1998; Maren, 1999). A single TBS to the EC induced a short-term synaptic enhancement of intracellular recorded postsynaptic potentials and extracellular recorded field potentials in the BLA that was consistent with our previously reported intracellular recordings obtained with a conventional high-frequency single-train stimulation (100 Hz for 1 s) (Li et al., 1998). In the presence of the 5-HT_2-receptor agonist DOI (100 μM), however, a single TBS induced a long-term synaptic potentiation lasting for more than 30 min. LTP obtained in this way requires NMDA-receptor activation because application of the NMDA-receptor antagonist, APV (100 μM), abolished the TBS-induced LTP even in the presence of the 5-HT_2-receptor agonist, DOI, whereas DOI alone had no observable effect on basal intracellular or field synaptic responses. The activation of 5-HT_2 receptors is essential for the facilitation effect of DOI on TBS-induced long-term synaptic potentiation, since both ketanserin and RS102221 were able to block the effect. This contrasts with the inhibitory effect of DOI on the induction of LTP observed in the visual cortex (Edagawa et al., 2000). This effect of DOI was blocked by the 5-HT_{2A}-receptor antagonist, ritanserin, and the $GABA_A$ antagonist, bicuculline, suggesting an involvement of GABAergic tone in the visual cortex (Edagawa et al., 2000).

Enhancement of NMDA-receptor functions by DOI

DOI showed a dose-dependent enhancement of NMDA potentials through a 5-HT_2 receptor-mediated mechanism. Activation of 5-HT_{2A} receptors by 0.1 μM (−)-1-2,5-dimethoxy-4-bromophenol-2-aminopropane (DOB) or 20 μM 5-HT markedly enhanced NMDA-induced inward current in neurons of the rat medial prefrontal cortex (Arvanov et al., 1999; Arvanov and Wang, 1998). A higher concentration of DOB (20 μM) in the same brain region suppressed NMDA-induced inward current (Arvanov et al., 1999). The biphasic effect of DOB was interpreted as an activation of protein kinase C (PKC) at low DOB concentration and calmodulin kinase II at high DOB concentration (Arvanov et al., 1999). Rahman and Neuman interpreted the same phenomenon as desensitization, but had to invoke more than one mechanism to explain its biphasic nature (Rahman and Neuman, 1993).

In the present study, 20–100 μM DOI induced only an enhancement of evoked NMDA receptor-mediated field

and intracellular recorded potentials in the amygdala. This effect was blocked both by the 5-HT_2 antagonist ketanserin and by the 5-HT_{2C} receptor selective antagonist RS102221. DOI treatment did not significantly affect basal calcium levels under our experimental conditions, although an increase in intracellular Ca^{2+} concentration was expected to occur and does occur as a result of 5-HT_2-receptor activation in glioma and NIH-3T3 fibroblastic cell lines (Kagaya et al., 1993; Price and Sanders-Bush, 2000). Taken together, our results indicate that 5-HT_2-receptor activation appears to facilitate NMDA channel function without causing a robust increase in calcium release from intracellular stores. A possible mechanism for 5-HT_2 receptor-mediated facilitation of NMDA-receptor responses is through activation of PKC. Both PKC and elevated intracellular calcium can activate proline-rich tyrosine kinase 2, which binds and phosphorylates the tyrosine kinase, Src (MacDonald et al., 2001). Src facilitates NMDA receptor responses by phosphorylating NR2A/2B subunits (Heidinger et al., 2002). The evidence further suggests that the receptor subtypes and/or signal transduction pathway involved in these phenomena may be different from that described in the frontal cortex (Arvanov et al., 1999). Such a cellular mechanism would allow for a fine adjustment of NMDA receptor-mediated functionality at synaptic boutons by serotonin corelease in amygdala.

PPF is considered to be a presynaptic phenomenon, resulting from a transient increase of presynaptic Ca^{2+} (Hess et al., 1987; Manabe et al., 1993; Schulz et al., 1994; Zucker, 1989). The PPF protocol has been used in the amygdala preparation by our laboratory and others to demonstrate a presynaptic mechanism in both excitatory and inhibitory terminals (Braga et al., 2002; Zinebi et al., 2001). In the present study, DOI enhanced the amplitude of NMDA receptor-mediated EPSPs associated with no significant change in the PPF ratio. This supports the conclusion that DOI acts predominantly on postsynaptic 5-HT_2 receptors to facilitate NMDA receptor-mediated synaptic responses. Furthermore, DOI did not significantly affect AMPA/kainate receptor-mediated synaptic components, which would not be true if 5-HT_2 receptors caused a facilitation of glutamate release. Collectively, the facilitating effects of DOI on NMDA receptor-mediated EPSPs and on NMDA-induced calcium influx of neuronal somata indicate predominantly postsynaptic mechanisms.

CONCLUSION

The present study provides the first demonstration that the activation of the 5-HT_2 receptor facilitates NMDA receptor-dependent synaptic plasticity in the BLA. NMDA receptor-mediated synaptic transmission and plasticity in the BLA play an important role in fear conditioning, emotional memory, kindling development and PTSD. Our data imply that activation of serotonergic input from the dorsal raphe can modulate NMDA receptor-dependent emotional learning and therefore regulate feelings of fear, anxiety and stress in response to external events. The results of this study provide a cellular basis for this regulation.

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APPENDIX 2

TWENTY

Psychosocial Stressors as Predisposing Factors to Affective Illness and PTSD

Potential Neurobiological Mechanisms and Theoretical Implications

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SENSITIZATION IN THE AFFECTIVE DISORDERS

Stressor and Episode Sensitization in the Unmedicated State

At the beginning of the twentieth century, Kraepelin (1921) laid out the fundamentals of the sensitization hypothesis of affective disorders:

> the attacks begin not infrequently after the illness or death of near relatives ... we must regard all alleged injuries as possibly sparks for the discharge of individual attacks, but the real cause of the malady must be sought in *permanent internal changes*, which at least very often, perhaps always, are innate ... in spite of the removal of the discharging cause, the attack follows its independent development. But, finally, the appearance of wholly similar attacks on wholly dissimilar occasions or quite without external occasion shows that even there where there has been external influence, it must not be regarded as a necessary presupposition for the appearance of the attack. (pp. 180–181)

In this terse and insightful paragraph, he outlines four different components of the sensitization hypothesis: (1) initial episodes of affective illness are often precipitated by psychosocial stressors; (2) as recurrences emerge, later episodes do not require the same psychosocial precipitation, but may occur more spontaneously; (3) episodes tend to occur with a characteristic similarity; and (4) innate neurobiological mechanisms mediate these vulnerabilities and recurrences, and presumably these could occur both on an inherited and an experiential basis.

Other aspects of this sensitization hypothesis are outlined in additional passages from his work. Although Kraepelin noted the "sheer immeasurable multiplicity of clinical pictures," (p. 114) and "the frequency, with which the different clinical forms of

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manic-depressive insanity here described occur in a fairly large series of observations, is naturally very various ..." (p. 133), "... for the most part the disease shows the tendency later on to run its course more quickly and to shorten the intervals, even to their complete cessation" (p. 137). "When the disease has lasted for some time and the attacks have been frequently repeated, the psychic changes usually become more distinct during the intervals also" (p. 149).

Thus, Kraepelin (1921) recognized both the inherent variability and unpredictability of bipolar episode presentation and course among and within individual patients; but within this seeming randomness, he noted a tendency for well intervals to decrease as a function of the successive number of episodes (i.e., sensitization) with a particularly striking effect after the first, second, and third episodes. Kraepelin recognized the poor prognostic implications of the occurrence of dysphoric mania and its high rate of hospitalization and chronicity, particularly in females. Herein he applied another postulate of the sensitization model, that with greater number of recurrences there may be a malignant progression and treatment resistance in the illness. We have summarized these essential elements of the sensitization hypothesis in Figure 20.1, with the more explicit hypothesis that greater numbers and/or faster cycling of episodes will be associated with greater treatment resistance, particularly to the classical modality of pharmacoprophylaxis – lithium carbonate.

Illness Progression During Tolerance Development

There is an additional component of the sensitization hypothesis in affective illness based on episodes breaking through previously effective pharmacoprophylaxis in a pattern that resembles tolerance. In these instances, patients who had previously been severely ill experience a good response to prophylactic monotherapy or combination therapy and remain well for a period of years, and then begin to experience breakthrough episodes of increasing severity or duration (Post et al., 1999; Post, Ketter, Denicoff, Leverich, & Mikalauskas, 1993; Post, Leverich, Rosoff, & Altshuler, 1990). These recurrences may progress to the point of complete loss of efficacy to what had previously been a highly effective treatment regimen.

A number of predictions in affective illness are based on a preclinical model of tolerance (Weiss, Clark, Rosen, Smith, & Post, 1995) to the anticonvulsant effects of mood stabilizing anticonvulsants on amygdala-kindled seizures (Table 20.1). These postulates, which remain to be further specifically examined for their clinical applicability, include:

1. A greater number of prior episodes (a marker of increased pathological illness drive) will be associated with a greater likelihood and more rapid onset of tolerance development;
2. Higher rather than minimally effective doses of a treatment will be more likely to prevent or delay tolerance development (for some, but not all drugs [e.g., with lamotrigine being a possible exception]);
3. Stable (or possibly descending) dose regimens will be preferable to minimally effective dosing followed by dose escalation in an attempt to treat breakthrough episodes;

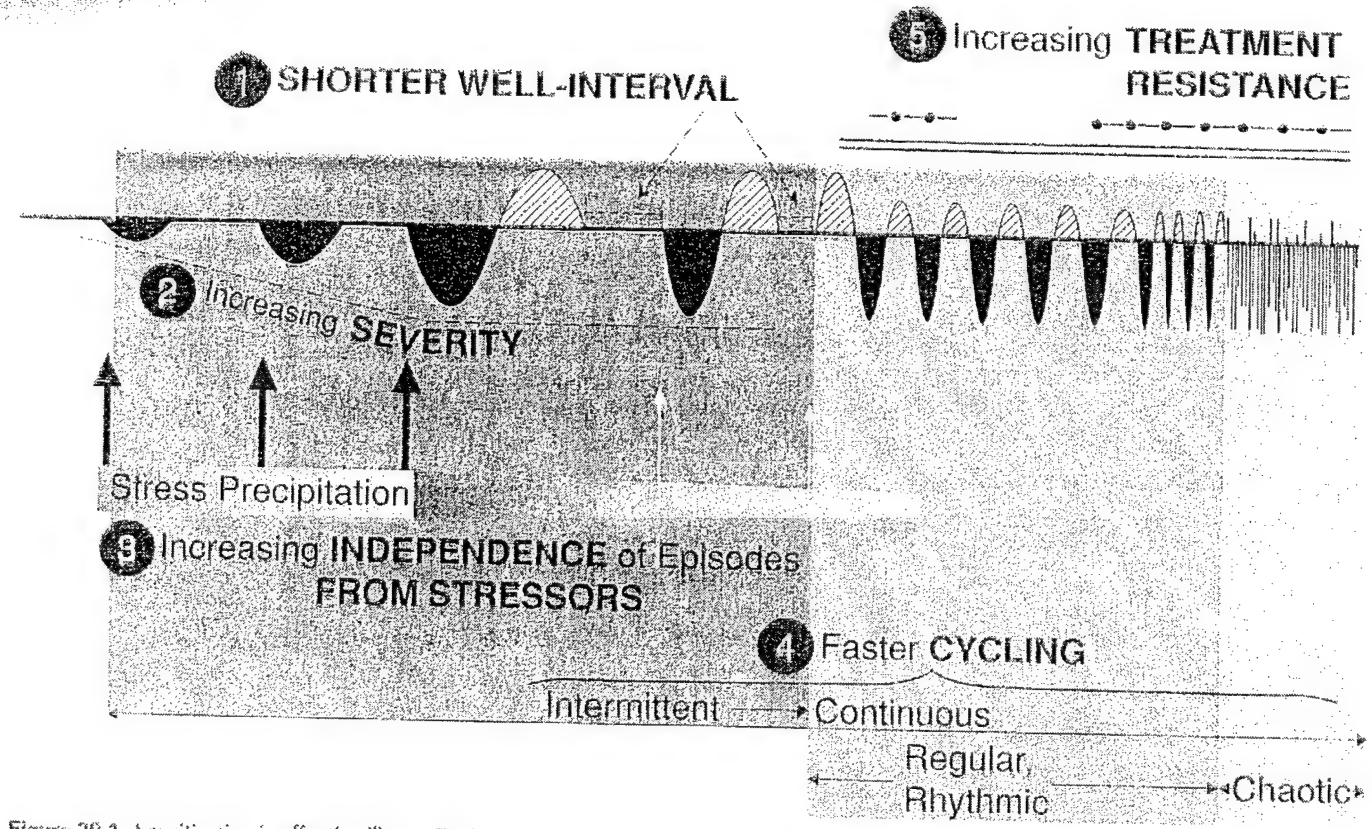


Figure 20.1. Sensitization in affective illness. Evidence of the tendency for the illness to progress is based on a variety of observations, including: (1) increases in episode frequency; (2) increases in episode severity, quality, or complexity; (3) early episodes precipitated by psychosocial stressors, but later ones occurring more spontaneously; (4) transition from intermittent to continuous to chaotic cycling patterns; and (5) possibly increasing treatment resistance, especially to lithium.

Table 20.1. Clinical Predictions[a] to Be Explored Based on Observations from a Preclinical Model of Amygdala-Kindled Seizures[b]

Tolerance to anticonvulsant effects slowed by:	Future studies; is there predictive validity for clinical tolerance in affective illness?
Higher doses (but lower doses w/LTG)	Maximum tolerated doses
Not escalating doses	Stable dosing
More efficacious drugs (VPA > CBZ)	Different rate of treatment resistance?
Treatments initiated early in illness	Sarantidis and Waters, 1981; Gelenberg et al., 1989; O'Connell et al., 1991; Denicoff et al., 1997; Swann et al., 1999
Combination treatment (CBZ plus VPA)	Combination > monotherapy?
Reducing illness drive	Treat comorbidities
Response restored by:	
Period of drug discontinuation then re-exposure	Randomized study of continuation treatment vs. discontinuation and re-exposure
Agents with different mechanisms of action (no cross-tolerance)	Cross tolerance from lamotrigine to CBZ, not VPA

[a] Right side of table.
[b] Left side of table.
VPA, valproic acid; CBZ, carbamazepine; LTG, lamotrigine.

4. Drugs with different mechanisms of action will have different potency in preventing tolerance development;
5. Combination treatments employing multiple and differential mechanisms of action will be more effective in delaying tolerance development than similar doses of several monotherapies;
6. When loss of efficacy via a tolerance mechanism has occurred, renewal of efficacy may be achieved by switching to or adding another drug with a different mechanism of action (i.e., one that does not show cross tolerance); and
7. In instances in which loss of efficacy has developed gradually via a purported tolerance mechanism, effectiveness of the initial drug may be re-achieved following a period of treatment with other agents and then reinstitution of the initial drug.

As noted below, some of these predictions based on the preclinical kindled seizure model have been preliminarily explored and partially validated, but a number remain to be directly tested in the clinic. Moreover, to the extent that these predictions of the model do prove valid, a third round of predictions could be derived based on presumed neurobiological and experiential effects on gene expression mechanisms involved. These also will be elaborated in a later section of the chapter.

Episode Sensitization

Kraepelin's empirical observations which form the basis of the sensitization model in affective illness have now been largely validated and confirmed by many investigators using a variety of study methodologies. Most studies support a greater role of

psychosocial stressors in initial, compared with later, episodes, with few exceptions (Table 20.2). Studies that have found that stressors continued to be associated with later episodes of affective illness have misinterpreted these observations as a refutation of the model (Hammen & Gitlin, 1997; Swendsen, Hammen, Heller, & Gitlin, 1995). This refutation is not true because the sensitization model is based on the assumption that over the course of illness, patients become *increasingly* sensitive to the role of stressors in the precipitation of episodes. To the extent that stressors are involved and documented, it supports the model; to the extent that later episodes are associated with or precipitated by symbolic stressors, conditioned stressors, or occur in the relative absence of exogenous stressors, this finding would also be consistent with the model. The model only suggests that there is a reduced need for the involvement of and direct triggering by stressors in later episodes.

Numerous studies support the general provision of cycle acceleration with shorter well intervals between successive episodes (Table 20.3), although, again, as Kraepelin and others have pointed out, there would be many individual exceptions to this rule. For example, we have observed a subgroup of patients who begin their illness with rapid or continuous cycling from the outset (Roy-Byrne, Post, Uhde, Porcu, & Davis, 1985) and, therefore, we would not expect any notable further degree of cycle acceleration; in fact, it might not be possible to demonstrate such a phenomenon in patients with continuous cycling from the outset because of a ceiling effect.

Moreover, Kraepelin (1921) made his observations in an era before major psychopharmacological interventions were available. Thus, many investigators who fail to observe this pattern or report that there is a lack of progression in the illness, or a failure of rapid cycling patients to continue in this pattern, have often not taken treatment into account (Coryell, Endicott, & Keller, 1992). Whenever effective treatment interventions are employed, the natural course of the illness would be altered, which is the goal of modern pharmacotherapeutic interventions. For example, Angst and Sellaro (2000) report that duration of the well interval only decreases over the first two episodes and then stabilizes thereafter.

Failure of well-treated patients to demonstrate this pattern of cycle acceleration and malignant progression of the illness cannot be taken as a refutation of the sensitization hypothesis. The fact that we and others have continued to observe this pattern of sensitization in treatment-refractory patients is predicated on their lack of adequate response to pharmacotherapy, presumably yielding a course and pattern of illness not entirely dissimilar from what might have been expected if they were untreated (as in the Kraepelinian era). It is also possible, however, that some treatment such as antidepressants could modify the course in an adverse fashion similar to that observed with levodopa treatment in Parkinson's disease, generating an increased rapidity of cycling of the on-off phenomenon (Nissenbaum et al., 1987).

The prediction that greater number of episodes prior to institution of lithium pharmacoprophylaxis is a negative prognosticator for response has also been widely replicated in the literature in two different types of studies. In the first type, studies overwhelmingly indicate that rapid cycling patients are less responsive to lithium than those without a rapid cycling pattern (Post, Ketter, Speer, Leverich, & Weiss, 2000; Post, Kramlinger, Altshuler, Ketter, & Denicoff, 1990). In addition, there is a considerable literature indicating that the number of episodes prior to institution of lithium

Table 20.2. Greater Association Between Life Events and First versus Subsequent Episodes of Affective Disorder

Author	Disorder	Number of Episodes	N	% Patients for Whom Major Life Events Preceded Episode		p Value	Assessment
				First Episode	Later Episode		
Matussek et al. (1965)	Depression	1	242	44		–	Stressors (138 psychologic; 58 somatic) had to clearly precede onset of episode
		2	135		34	–	
		3	82		24	–	
		4	119		19	–	
Angst (1966)	Depression	1	103	60		–	No inventory
		≥ 4			38	–	
Okuma and Shimoyama (1972)	Manic Depression	1	134	45		–	Any event (3 months prior)
		2	134		26	–	
		3	134		13	–	
Glassner et al. (1979)	Manic Depression	1	25	75		–	Event rated stressful by patient and on Holmes and Rahe Scale (1 year prior; usually 2–24 days); role loss critical in patients and comparison subjects
		≥ 1[a]			56		
Ambelas (1979)[b]	Mania	1	14	50		< 0.01	Paykel Life Events Scale (4 weeks prior); one-third of cases followed bereavement
		≥ 2		67	28		
Ayuso et al. (1981)	Depression	1	43	55.8		< 0.05	Social and somatic stressors; patients with late onset had more events than did those with early onset
		2	35		40.0		
		3	18		38.8		
		≥ 4	47		29.7		
Perris (1984)	Depression	1	37	62	50[c]	< 0.02	Semistructured interview; 56 item inventory (3 months prior)
		≥ 2	112	43	19[d]	< 0.001	

Dolan et al. (1985)	Depression	1	21	62		< 0.05	Bedford College-Life Events and Difficulties Schedule (6 months prior) (Brown, Harris, 1978)
		≥ 2	57		29		
Ezquiaga et al. (1987)	Depression	< 3	52	50		< 0.01	Semistructured interview (Brown, Harris); no effect on chronic stress
		≥ 3	45		16		
Ambelas (1987)	Mania	1	50	66		< 0.001	Paykel Life Events Scale (4 weeks prior)
		≥ 2	40		20		
Ghaziuddin et al. (1990)	Depression	1	33	91		< 0.05	Paykel Life Events Scale (6 months prior)
		≥ 2	40		50		
Cassano et al. (1989)	Depression	1	94	66.0		< 0.05	Paykel Life Events Scale
		≥ 2	173		49.4		
Hammen & Gitlin (1997)	Bipolar	0–8	52	40		0.05	More episodes, more stressors and relapsed faster
		≥ 9			76		
Castine et al. (1998)	Schizophrenia	≤ 3	32	more recent life events		0.01	Paykel Life Events Scale
Nierenberg et al. (1998)	Depression	1st vs. > 3 episodes	176	1st episode had more stressful negative life events compared with recurrent		0.037	Life Events Scale, Perceived Stress Scale

[a] For this group, the most recent hospitalization was preceded by a life event resulting in role loss.
[b] Of surgical comparison subjects, 6.6% had experienced recent major life events.
[c] Percentage for negative or undesirable events.
[d] Percentage for events involving psychological conflict.

Table 20.3. Early Studies of Life Course of Manic-Depressive Illness

Study	# of pts.	High UP/BP Pt. Ratio	Sex	Age of Onset (Peak or Mean Years)	Decreasing Well Interval	Observational Time (years) Retrospective (R) Prospective (P)	Late Age at Onset Predicts Increased Relapse	Comments
Swift (1907)	105	No	74 F 31 M		Yes	R	Yes	Study examined first episode in terms of prognosis. Prognosis is better if first episode is a depression than if it is mania.
Kraepelin (1921)	903	Yes	648 F 255 M	20–30	Yes	Variable up to a lifetime R,P	Yes	Study contained both a large number of patients and extended periods of observation. Few patients were observed for their complete life course.
Malzberg (1929)	11,393		6513 F 4880 M	mean = 40			Yes	Study examined only first admissions and reviewed relationship to duration of episode and recovery.
Paskind (1930)	633			21–30	Yes	R	Yes	Nonhospitalized patients studied – may be helpful as a comparison group to the hospitalized patients. *Late age of onset predicts increased duration of episodes.
Pollock (1931)	8438	No	519 F 3274 M	20–24		11 R	Yes	Study examined a large number of patients. Many unrecovered cases were discharged as improved. Age of onset between 20 and 40 years – better prognosis than patients younger or older.
Steen (1933)	493			20–40		8 R	Yes	Study confused other diagnostic groups (i.e., schizophrenia, schizoaffective) with BP patients. Twenty to 30-year age of onset predicts high rate of recovery.

Rennie (1942)	208	Yes	117 F 91 M	45–55		20 R		Seventy-nine percent of patients will have more than 1 episode during their lifetime; 50% will have less than 3 episodes; 93% (193) recovered from first episode; 21% (62) never had another recurrence.
Poort (1945)	141	No		20–30		10–15 R	No	Fifty percent (71) were recurrent UP or BP; 19% (27) went on to develop another type of psychosis such as schizophrenia, "hysteria," or sociopathy.
Lundqvist (1945)	319		196 F 123 M	< 30/mania > 50/depression	Yes	14–32 R	Yes	Patient population is difficult to assess because 28% (89) became chronic following their first episode and 7% (22) developed schizophrenia. First episode of mania predicts increased risk for relapse.
Stenstedt (1952)	216	Yes	126 F 90 M	mean = 38.7 F		29 R		Study does not detail polarity of episodes in UP and manics who relapse; 11.7% was the morbidity risk of the illness among siblings and children of probands; 83% (117) had first episode as a depression; 53% (114) had one episode.
Astrup et al. (1959)	270	Yes				5–19 R	No	Study separates schizoaffective from manic-depressive illness; emphasizes the need for long-term follow-up to make separation

(continued)

Table 20.3 (*continued*)

Study	# of pts.	High UP/BP Pt. Ratio	Sex	Age of Onset (Peak or Mean Years)	Decreasing Well Interval	Observational Time (years) Retrospective (R) Prospective (P)	Late Age at Onset Predicts Increased Relapse	Comments
Angst and Weiss (1967)	388	Yes		mean = 38.5 (BP)	Yes	7 R	Yes	Study clarifies set of definitions for episode, interval, cycle. Confirms by use of statistics the earlier observations of the relationship of age of onset and number of episodes with prognosis. Only 12% (45) of patients were BP type.
Bratfos and Haug (1968)	207	Yes	116 F 91 M	mean = 35	Yes	6 P	No	Patients had various types of somatic therapy (ECT, antidepressant, and neuroleptics). Study did not distinguish between the type of therapy received in terms of risk for relapse; 20% (41) remained chronically ill.
Perris (1968)	270	No	144 F 126 M	mean = 37.7		20 R		Eighty-four percent of UP patients will convert to BP illness before 3 episodes, i.e., 16% of BP patients will be misdiagnosed as UP with up to 3 observations of depressive episodes. BP patients are at higher risk for relapse than UP.
Grof et al. (1974)	987	Yes			Yes	Up to 45	Yes	Patients were treated only during the acute phases of their illness and not prophylactically. Each succeeding cycle length is shorter on the average than the preceding one.

Taschev (1974)	652	Yes	350 F 323 M		Yes	R	Yes	Retrospective evaluation of cyclothymic depression (122, 18.7%), recurrent depression (134, 20.5%), involutional depression (335, 51.3%), reactive depression (23, 3.5%), recurrent manla (38, 5.8%); 26.4% (172) of depressives committed suicide; 16% (104) became chronic; no mention if patients were treated.
Angst et al. (1978)	254	Yes				12–16 P		Study of the number of episodes before conversion of UP or BP. Conversion of UP to BP > 3 episodes = 70%; > 6 episodes = 83%. Conversion of BP to schizoaffective: 3 of 40 (7.5%).
Angst (1978)	95	No	58 F 37 M	mean = 61		26 R, P		Statistical description of long-term observation of BP illness. Study examines the heterogeneity of BP illness. Females exhibit more depression than mania. Males exhibit a symmetrical distribution of mania/depression.
Zis et al. (1980)	334	Yes			Yes		Yes	Increased risk of relapse is a function of number of previous episodes.
Maj et al. (1992)	72	Yes	42 F 30 M	mean = 32.6	Yes	P	No	Increasing severity from the index episode to the first, second, and third prospective episodes.
Angst and Sellaro (2000)	549	No			Yes	329 R 220 P	No	Increased rapidity of episode recurrence only after the first 2 episodes.

Key: UP = Unipolar; BP = Biploar; ECT = Electroconvulsive Therapy.

Table 20.4. More Episodes Prior to Starting Lithium Is Associated with Poor Prophylactic Response

Investigator	Correlates of Poor Response to Lithium
Prien et al., 1974	High frequency of hospitalizations
Sarantidis and Waters, 1981	More episodes per year
Abou-Saleh and Coppen, 1986	Higher number of episodes (7.0 ± 1.3) in bipolar patients
Gelenberg et al., 1989	> 3 prior episodes
O'Connell et al., 1991	$\geq$ 3.8 mean episodes
Goldberg et al., 1996	$\geq$ 2 prior hospitalizations
Denicoff et al., 1997	• Older age at first treatment • Longer duration of illness • More than 1 hospitalization for mania
Maj et al., 1998	$\geq$ 7.2 mean episodes
Swann et al., 1999	$\geq$ 10 prior episodes

prophylaxis is a negative predictor of lithium response (Table 20.4). Of course, it is also possible in these uncontrolled studies that a greater number of episodes is only a marker for a subsequent adverse course that would have been manifest even if lithium prophylaxis were instituted after the first or second episode.

Although the study of Kessing, Bolwig, and colleagues (1998) involving more than 20,000 patients in the Danish Case Registry did not initially control for treatment, it is striking that they found a direct relationship between the number of prior episodes and both the incidence of and latency to relapse into another episode in unipolar and bipolar patients. These data from a country in which lithium is very widely used provide some of the strongest evidence of an overall sensitization effect, that is, greater number of prior episodes is associated with a greater risk of relapse in both of these affective disorders. This trend apparently emerges irrespective of whether or not patients were treated in the community with sustained prophylactic medication (although the direct analysis of this inference remains to be reported).

Stressor and Episode Sensitization

In another seminal study, in more than 600 female identical twin pairs, Kendler et al. (1993) showed that strong predictors of major depression included both stressors and number of prior episodes. They also documented that a variety of early life stressors such as lack of parental warmth and parental loss were associated with the onset of initial or minor (neurotic) depressions and that more concurrent psychosocial stressors were involved in the precipitation of recurrent episodes. Thus, these data appear to support an early vulnerability factor of the environment, perhaps leaving the patient at a higher risk for subsequent stressor-related induction of depression. Kendler et al. also found that neurotic or minor depression predisposed to major depression and prior major depressive episodes predisposed to further depressions. A genetic component accounted for part of the variance in both the initial and recurrent episodes.

Thus, there is substantial evidence for two types of sensitization: (1) most prominently, early childhood and subsequent adult life stressors can act as precipitants or vulnerability factors (stressor sensitization), and (2) episode sensitization, in which the number of prior episodes correlates with the likelihood of relapse and a shortening of

Table 20.5. Sensitization Phenomena in Affective Illness

Observation	Evidence	Investigators
↑ FREQUENCY OF RECURRENCE		
Well interval	+++	Kraepelin; Grof; Post
↑ SEVERITY		
As function of episode number	+	Maj
↑ CHRONICITY		
Each new episode carries added 10% risk of nonrecovery	++	Thase
↑ TREATMENT RESISTANCE		
First major depression more responsive than second	+	Angst
Greater number and frequency of prior episodes associated with lithium nonresponse	+	Gelenberg; O'Connell; Denicoff
↑ ABNORMAL NEUROBIOLOGY		
More Episodes:		
– More sleep abnormalities	++	Armitage; Thase
– Greater hypercortisolism	+	Ribeiro; Gurguis

the well interval. Preliminary data (Table 20.4) also suggest greater treatment refractoriness as a function of number of prior episodes.

Given these two major perspectives of the model (i.e., stressor and episode sensitization), it is then necessary to ascertain which underlying neurobiological mechanisms could mediate such long-term vulnerability from early stressors and episodes of affective illness themselves. Kraepelin (1921) wrote "... the real, the deeper cause of the malady is to be sought in a permanent morbid state which must also continue to exist in the intervals between the attacks" (p. 117). Although there are only a modicum of data available on the neurobiological correlates of episode sensitization in man, as outlined in Table 20.5, there is a very considerable preclinical literature that at least provides a basis for examining their potential relevance to the clinic.

There is considerable evidence for cross-sensitization between some types of stressors and cocaine administration, suggesting that elements of cocaine sensitization may parallel phenomena observed in stressor sensitization (Antelman, Eichler, Black, & Kocan, 1980; Kalivas & Duffy, 1989; Post, Ketter, Speer, Leverich, & Weiss, 2000). However, episodes of cocaine-induced hyperactivity and stereotypy can, in addition, serve as models of brief episodes of manic-like hyperactivity and frantic psychomotor drive that are not unlike those that occur in some patients with mania and dysphoric mania, respectively. As such, cocaine sensitization may be examined from the perspective that it could model aspects of both stressor sensitization and manic episode sensitization, and direct one toward the examination of whether some parallel neurobiological mechanisms are involved in all three types.

Sensitization and Kindling Phenomena Differences

We will only briefly allude to the neurobiology of another model of long-lasting neuronal learning and memory – amygdala kindling, which differs considerably (in behavior, biochemistry, neural pathways, and pharmacology involved) from sensitization

(Weiss & Post, 1994). The kindling model is an interesting model not only for examining the development of epileptogenesis, but also for observing lasting changes in neural and behavioral responsivity in response to very brief periods of brain stimulation. In amygdala kindling, repeated administration of subthreshold amygdala stimulation evokes afterdischarges of increasing frequency, duration, and complexity which then spread throughout the brain, involving other limbic and cortical structures in association with the development of increasingly robust and complex behavioral phenomena, culminating in a full-blown generalized seizure (Goddard, McIntyre, & Leech, 1969; Racine, 1972a, 1972b). Following sufficient numbers of amygdala stimulations associated with completed kindled seizures, a phase of spontaneity may emerge in which animals exhibit true epilepsy and have spontaneous seizures in the absence of exogenous physiological stimulation.

Thus, the kindling model provides a readily identifiable set of physiological and behavioral concomitants of neuronal learning and memory, each of which demonstrates an obvious augmented response to repeated brain stimulation and then culminates in a further progression to spontaneous episodes. At the level of both physiology (amygdala excitability thresholds and afterdischarges as well as their spread to other brain areas) and behavior (seizure stage evolution from partial to full-blown and then to spontaneous), increases in responsivity occur.

Although these electrophysiological and behavioral progressions present clear evidence for neuronal learning and memory phenomena, it is equally clear that they do not represent endpoints directly parallel to those that occur in bipolar illness (Weiss & Post, 1994). Complex partial seizures of the temporal lobe are often associated with prominent affective symptoms, but, conversely, primary affective illness is rarely associated with seizure-like manifestations. Moreover, the induced seizures of electroconvulsive therapy are used as a prominent treatment for severe manic and depressive episodes. Thus, kindling to a seizure endpoint must be considered a nonhomologous model for the affective disorders, since neither the inducing phenomena, nor the behaviors or their temporal domains, nor the pharmacological interventions between the two are identical or even highly similar.

However, kindling may be useful in understanding how a complex behavioral phenomenon such as a major motor seizure comes to be evoked by previously subthreshold stimuli upon repetition and, as well, how such precipitated episodes may proceed to the spontaneous variety. In addition, the kindled seizure model is particularly appropriate for examining tolerance development to the anticonvulsant effects of a variety of effective antiepileptic agents (Weiss, Clark, Rosen, Smith, & Post, 1995) many of which are now also used for the treatment of affective disorders (Dunn et al., 1998; Post et al., 1996, 1998a, 1998b). One can examine the principles and underlying neural mechanisms of tolerance development to these agents in the seizure realm and ask whether or not similar phenomena exist for tolerance development in other models or clinical situations, such as the tolerance that can occur in the prevention of paroxysmal pain syndromes (Pazzaglia & Post, 1992), migraine headaches (Post & Silberstein, 1994), breakthrough panic attacks, and most pertinent to the current discussion, episodes of recurrent affective disorder (Post, Ketter, Denicoff, Leverich, & Mikalauskas, 1993; Post & Weiss, 1996).

Thus, the stressor and cocaine sensitization models have direct parallels and homologies to phenomena that occur in the clinical realm in the course of unipolar and

bipolar affective disorders, whereas kindling must be considered only as an analogy and used for its indirect parallels. The kindling model's degree of predictive validity in this indirect realm remains to be determined, but will in turn reveal its ultimate utility.

NEUROBIOLOGICAL MECHANISMS FOR LONG-LASTING BEHAVIORAL AND BIOCHEMICAL VULNERABILITIES FOLLOWING EARLY LIFE STRESSORS

Levine et al. (1991) have used a paradigm of a single 24-hour period of maternal deprivation in rat pups as an inducer of long-lasting altered behavior and biochemical responsivity. These animals, like those of Plotsky and colleagues (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999; Ladd et al., 2000; Plotsky, 1997) which are subjected to repeated episodes of three hours of maternal deprivation in the first weeks of life, show long-lasting hypercortisolism and increased anxiety-like behaviors.

Neurobiology of Repeated Maternal Separation: Parallels to the GR Knock-out Mouse

The studies of Plotsky and Meaney are particularly interesting from the perspective of adaptive and homeostatic mechanisms, because animals subjected to only fifteen minutes of daily maternal separation are protected against age-related loss of hippocampal anatomy and associated decline in learning and memory skills (Anisman, Zaharia, Meaney, & Merali, 1998; Liu et al., 1997; Meaney, Aitken, Van Berkel, Bhatnagar, & Sapolsky, 1988). Meaney has demonstrated that the mechanism of this effect is an increase in maternal licking behavior that occurs following the fifteen-minute separation, but not after a three-hour separation. After three hours, the previously separated pups are apparently not well identified and maternal behavior is degraded, with increased agitation in the mother and, in some instances, apparent frantic trampling of her offspring. It appears that this element of maternal behavior and neglect is a crucial element in producing the long-lasting hypercortisolism and anxiety-like behaviors in the separated offspring. This behavior can be remedied or prevented if the mother is given substitute rat pups during the three-hour period of separation from her own pups. In this case, when the separated pups are returned, the mother's behavior is normal and no lasting behavioral or biochemical alterations in the offspring are produced (Meaney, 1999).

In the three-hour separated pup that receives the full separation/maternal malbehavior, it is remarkable that these animals as adults show an increased proclivity to self-administer alcohol and cocaine compared with litter mates without such early stressors (Meaney, Brake, & Gratton, 2002; Huot, Thrivikraman, Meaney, & Plotsky, 2000). The entire biobehavioral syndrome, including hypercortisolism, is reversed by serotonin-selective antidepressants. However, if the antidepressant drug treatment is discontinued, animals revert to their prior anxious and hypercortisolemic state.

This model thus provides a dramatic illustration of how experiences in the environment can induce lasting behavioral and neurobiological changes at the level of gene expression. Increases in corticotropin-releasing factor (CRF) mRNA, for example, have recently been demonstrated in the hypothalamus of these animals (Francis, Caldji, Champagne, Plotsky, & Meaney, 1999). Moreover, the ability of these early stressors to alter the expression of these behavioral and biochemical changes in a life-long fashion parallels a related syndrome that can be induced genetically using transgenic animals.

For example, Barden and colleagues (Pepin, Pothier, & Barden, 1992) have developed a strain of animals with deficient glucocorticoid receptor number that also have resultant hypercortisolemia and increases in anxiety and depressive-like behaviors. These behaviors can also be reversed by treatment with antidepressant medications (Beaulieu, Rousse, Gratton, Barden, & Rochford, 1994; see also Figure 20.2). Thus, these two different animal models of life-long hypercortisolemia and lasting behavioral aberrations illustrate that hereditary genetic as well as experiential alterations in gene expression based on environmental insults, can each be sufficient to induce fairly similar phenotypic syndromes.

Neurobiology of One-Day Maternal Separation in the One-Day-Old Rat Pup

Further insight into the way isolated stressful life events occurring early in an animal's development could produce temporary to long-lasting neurobiological alterations is revealed by studies with the single-day separation model (Levine, Huchton, Wiener, & Rosenfeld, 1991). Zhang et al. (2002) have demonstrated a doubling of apoptosis (preprogrammed cell death) in widely distributed cells in the brain of separated rat pups after maternal deprivation. Double staining techniques show that this involves both neurons and glia.

In white matter areas of brain, the degree of induction of nerve growth factor (NGF) as measured by *in situ* hybridization of mRNA is directly proportional to the degree of apoptosis (Zhang, Xing, Levine, Post, & Smith, 1998). This apparently paradoxical finding of increased neurotrophic factor gene induction associated with greater degrees of apoptosis is understandable from the perspective that the NGF receptor trk A is not fully developed at this time and NGF potently binds instead to the lower affinity p75 receptor component, which is an executor of a death program rather than a neurotrophic one typical of the trk A receptor (Barrett, 2000). These data are thus of considerable interest in suggesting that mal-timed induction of neurotrophic substances may be as harmful as their deficiency of expression in other instances.

The one-day separation stress is also associated with increased expression of the proto-oncogene c-fos mRNA and the cell death factor Bax mRNA. In addition, in the hippocampus, there is decreased gene expression of calcium calmodulin kinase-II (CaMKII), the growth factor brain-derived neurotrophic factor (BDNF), and the inducible form of nitric oxide synthase (iNOS) (Xing et al., 1998, and unpublished data, 2000). These substances are of potentially great interest in relation to their important roles in long-term potentiation and other models of learning and memory. In this regard, the decrease in neurotrophic factors and increase in cell death factors following the one-day separation stress could be pertinent to findings that some individuals with posttraumatic stress disorder (PTSD) may have decreased volume of their hippocampus assessed by magnetic resonance imaging (MRI) (Bremner et al., 1995, 1997; Gurvits et al., 1996) and an associated impairment in learning and memory. However, the cause and effect relationships in these instances remain to be elucidated (Bremner, 2001).

Although there has been no direct pathophysiological link between decrements in BDNF (or any other neurotrophic factor) and the size of the hippocampal alterations in learning and memory in this paradigm, the ability of stressors to decrease BDNF at least provides a potential explanatory mechanism that can be further examined for

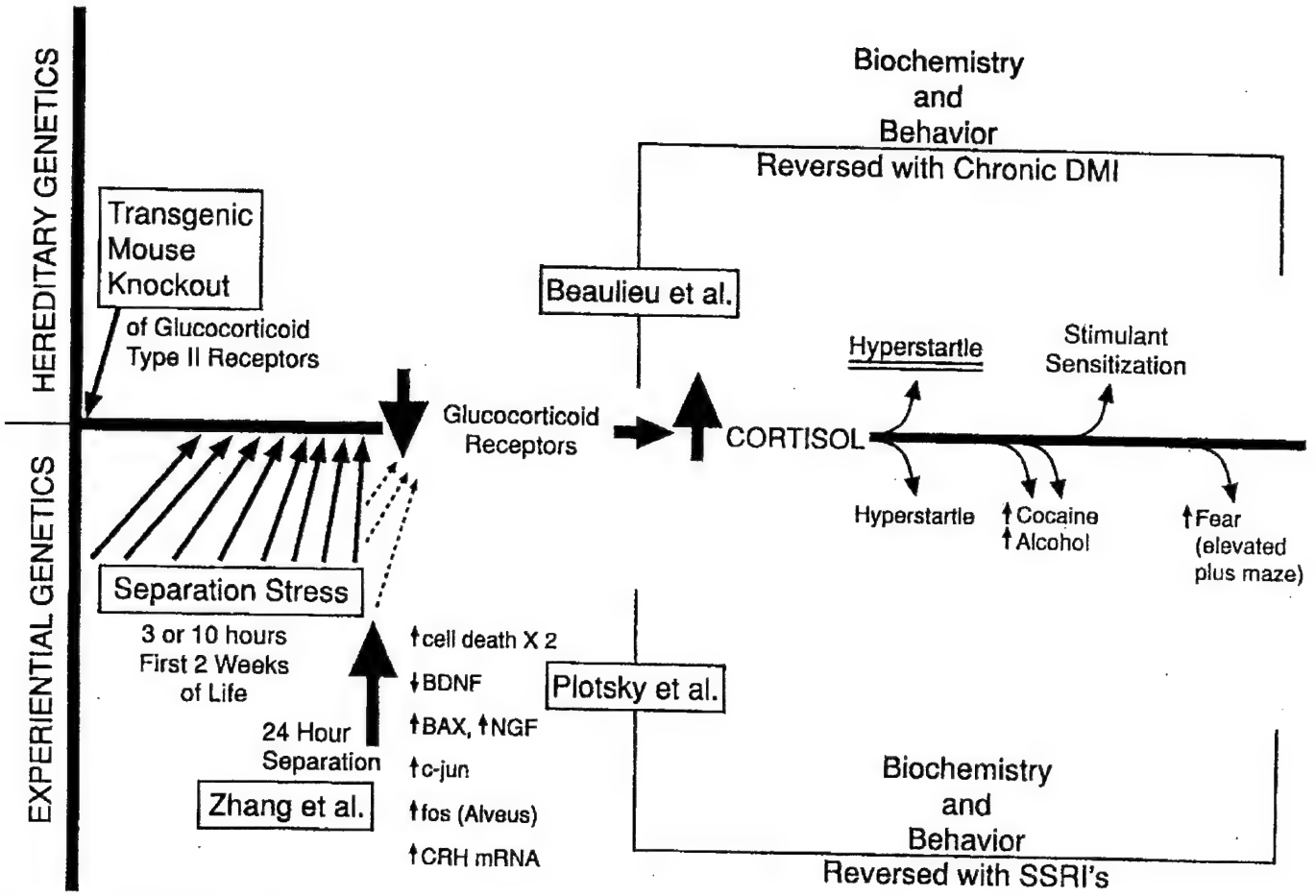
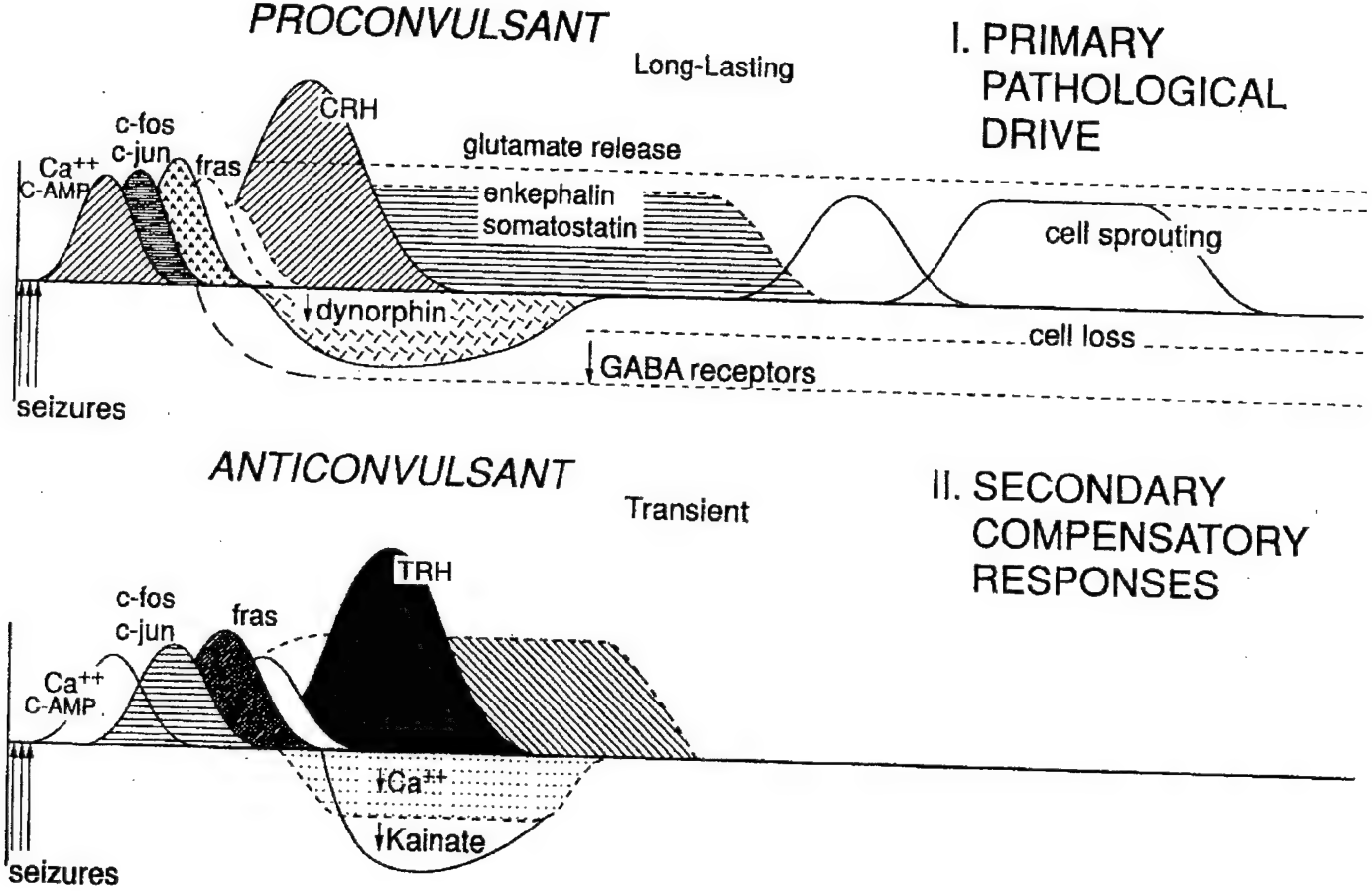


Figure 20.2. Convergent genetic and environmental models of depression. Either heredity or experiential genetics may lead to compounding behavioral and biochemical end points similar to those seen in depression and reversible by antidepressants. DMI, desipramine; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; CRH, corticotropin-releasing hormone; SSRIs, serotonin selective reuptake inhibitors.


PROCONVULSANT
Long-Lasting
I. PRIMARY PATHOLOGICAL DRIVE
CRH
c-fos
c-jun
fras
Ca++
C-AMP
glutamate release
enkephalin
somatostatin
cell sprouting
dynorphin
cell loss
GABA receptors
seizures
ANTICONVULSANT
Transient
II. SECONDARY COMPENSATORY RESPONSES
TRH
c-fos
c-jun
fras
Ca++
C-AMP
Ca++
Kainate
seizures

its putative role in such a relationship. Supporting the possibility that BDNF could be a crucial factor in learning and memory defects are the observations of Korte et al. (1998) indicating that transgenic animals with an absence of BDNF have both deficient long-term potentiation (LTP), and an inability to navigate accurately in the Morris maze test, indicating a deficit in the ability to perform normal tasks that are ordinarily well within the animal's normal repertoire.

One is now in a position to ask questions such as what are the crucial degrees of BDNF, CaMKII, or iNOS decrement that might be etiopathological to the observed behavioral and biochemical alterations. We would imagine that there is a considerable range of different parameters that may influence the ultimate impact and outcome of a stressor, including severity, duration, quality, and timing, as well as the number of repetitions and recurrences later in development. The degree of both genetic vulnerability and stressor resistance or resilience (Luthar, Cicchetti, & Becker, 2000) in conjunction with the potential for adaptation to the stressor and the support provided by others (Breier et al., 1988) may all be crucial variables in whether long-term pathological neurochemical and behavioral alterations become manifest.

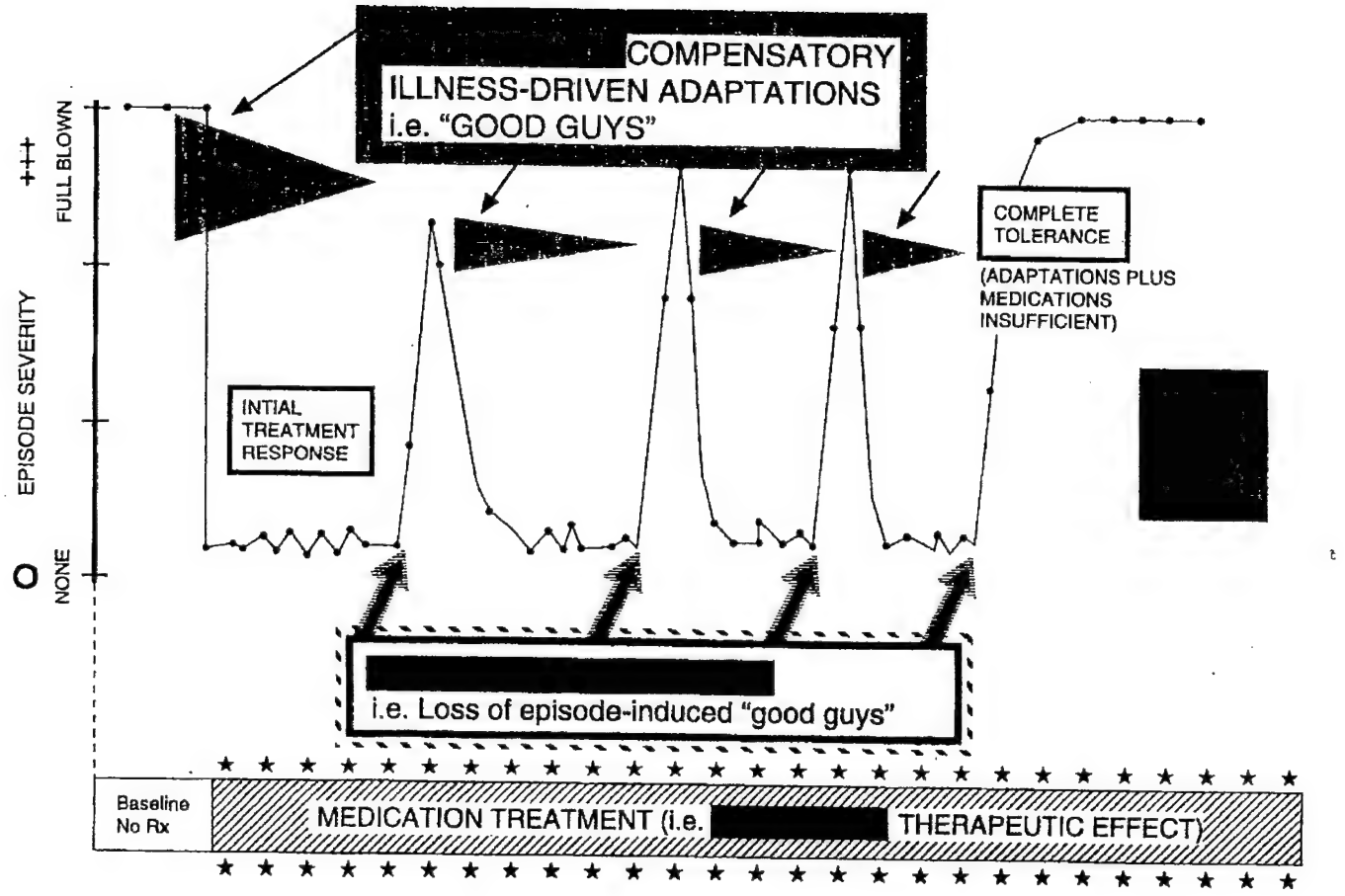
Pathological versus Adaptive Alterations in Gene Expression in Kindled Seizures

It is perhaps useful to discriminate between changes in gene expression that are related to the primary pathology of sensitization versus those that are compensatory and adaptive. A similar distinction is more readily identifiable for amygdala-kindled seizures. Here we have provisionally divided the many changes in gene expression into these two components (Figure 20.3). One is based on whether they are primary and important to the maintenance of the kindled "memory" trace, or whether they reflect endogenous anticonvulsant adaptations that attempt to return the animal to homeostasis (Post & Weiss, 1992, 1996).

This separation has potential clinical importance from a number of perspectives, perhaps the most significant of which is that it provides new, dual, and differential targets of therapeutics. One can attempt to both block the primary pathological changes of kindling progression and, conversely, enhance the endogenous anticonvulsant or adaptive processes.

We have preliminarily identified thyrotropin-releasing hormone (TRH) as an endogenous anticonvulsant substance because it transiently increases in the hippocampus after seizures and has been reported to be anticonvulsant (Kubek, Liang, Byrd, & Domb, 1998). We have extended these observations with the finding that intra-hippocampal injection of TRH suppresses amygdala-kindled seizures (Wan, Noguera, & Weiss, 1998).

Figure 20.3. Schematic illustration of potential genomic, neurotransmitter, and peptidergic alterations that follow repeated kindled seizures. Putative mechanisms related to the primary pathological drive (i.e., kindled seizure evolution) are illustrated on top and those thought to be related to the secondary compensatory responses (i.e., anticonvulsant effects) are shown on the bottom. The horizontal line represents time. Sequential transient increases in second messengers and immediate early genes (IEGs) are followed by longer lasting alterations in peptides, neurotransmitters, and receptors or their mRNAs, as illustrated above the line, whereas decreases are shown below the line. Given the potential unfolding of these competing mechanisms in the evolution of seizure disorders, the question arises regarding whether parallel opposing processes also occur in the course of affective illness of other psychiatric disorders. Endogenous adaptive changes (bottom) may be exploited in the design of the new treatment strategies.


COMPENSATORY
ILLNESS-DRIVEN ADAPTATIONS
i.e. "GOOD GUYS"
COMPLETE
TOLERANCE
(ADAPTATIONS PLUS
MEDICATIONS
INSUFFICIENT)
INTIAL
TREATMENT
RESPONSE
EPISODE SEVERITY
+++
FULL BLOWN
0
NONE
i.e. Loss of episode-induced "good guys"
Baseline
No Rx
MEDICATION TREATMENT (i.e.
THERAPEUTIC EFFECT)

This is of additional interest because, in animals that have become tolerant to the anticonvulsant effects of either carbamazepine or diazepam, seizures that normally increase TRH mRNA no longer do so (Weiss, Clark, Rosen, Smith, & Post, 1995). It is postulated that the failure to induce some compensatory adaptive changes in gene expression (such as TRH, $GABA_A$ receptors, etc.) may be intimately involved with the tolerance process. This would also provide a conceptual mechanism for how the therapeutic efficacy of a drug may be revived after tolerance has developed following a period of time off the drug (which would theoretically allow seizures to again induce the TRH mRNA and other adaptive changes in gene expression) and thus facilitate carbamazepine's effectiveness.

Pathological versus Adaptive Changes in Gene Expression in Affective Disorders?

Not only have we postulated that the relative balance and predominance of primary pathological compared with the secondary and adaptive alterations in gene expression are associated with the development of loss of efficacy to some classes of anticonvulsants in the tolerance model, but this ratio might also be relevant in determining, in the medication-free state as well, whether seizures are manifest or not during kindling development and expression. Do parallels exist in the progressive evolution of illness in other syndromes? In this regard, we would surmise that the increases in CRF (in turn driving increases in cortisol) may be representative of one of the primary pathological processes of depression evolution and progression, particularly since hypercortisolemia has been associated with depression and cognitive impairment in other syndromes, such as Cushing's disease (Starkman, 1993).

Conversely, the increases in TRH directly reported in cerebrospinal fluid (CSF; Banki, Bissette, Arato, & Nemeroff, 1988) or inferred from the associated blunting of TSH response to TRH (suggestive of subclinical hyperthyroidism; Loosen, 1985), may be part of an endogenous set of antidepressant mechanisms that help to naturally terminate a depressive episode. Thus, as schematized in Figure 20.3, we again hypothesize that it is the ratio of the primary pathological versus secondary adaptive changes in gene expression that determine whether an individual remains in a relatively euthymic state or proceeds toward the recurrence of further episodes of affective disorder (Figure 20.4).

Figure 20.4. Hypothetical schema of the role of endogenous regulatory factors in the generation and progression of illness cyclicity. After an illness episode, adaptive compensatory mechanisms are induced (i.e., "good guys"; shaded triangle with two stars), which together with drug treatment suppress the illness (initial treatment response; box). The "good guys" dissipate with time (i.e., the time-off seizure effect), and episodes of illness re-emerge. Although this re-elicits illness-related compensatory mechanisms, the concurrent drug treatment prevents some of the illness-induced adaptive responses from occurring (smaller triangles with one star). As tolerance proceeds (associated with the loss of adaptive mechanisms), faster illness re-emergence occurs. Thus, the drug is becoming less effective in the face of less robust compensatory mechanisms. The primary pathology is progressively re-emerging, driven both by additional stimulations and episodes (i.e., the kindled memory trace, or the "bad guys") along with a loss of illness-induced adaptations. Because this cyclic process is presumably driven by the ratio of the "bad vs. good guys" at the level of changes in gene expression, we postulate that such fluctuations in the "battle of the oncogenes" arising out of illness and treatment-related variables could account for individual patterns in illness cyclicity.

Table 20.6. Incidence of Traumatic Stressors in Bipolar Patients in the Stanley Foundation Bipolar Network

	Child *N*(%)	Adolescent *N*(%)	Adult *N*(%)
Physical Abuse			
Not Abused	228 (76)	242 (80)	246 (88)
Abused	71 (24)	59 (20)	37 (12)
Sexual Abuse			
Not Abused	241 (76)	247 (83)	242 (82)
Abused	59 (20)	51 (17)	55 (18)

IMPACT OF EARLY STRESSFUL EXPERIENCES IN BIPOLAR AFFECTIVE DISORDER

Although a number of studies outlined in Table 20.2 have examined the relative role of stressors in initial versus later episodes of both unipolar and bipolar depression, there has been less examination of the impact of early stressful life events on the subsequent course of bipolar illness. In the Stanley Foundation Bipolar Treatment Outcome Network, which now follows more than 500 patients on a detailed daily basis with the NIMH-LCMp (prospective version of the life chart method) (Leverich et al., 2001), we had the opportunity to address this question in 631 consecutive outpatients who completed a detailed questionnaire which included items related to whether or not they were exposed to physical or sexual abuse in childhood or adolescence (Leverich et al., 2002).

The incidence rate of these types of extraordinary psychosocial stressors listed in Table 20.6 is parenthetically and disappointingly not that different from the rate observed in many unselected nonclinical populations. However, in the context of patients with bipolar illness, the reported occurrence of either early physical or sexual abuse in childhood or adolescence was highly associated with an earlier onset of affective illness and more rapid, ultrarapid, and ultradian cycling patterns (Table 20.7). In the univariate analyses subjected to Bonferroni correction and in the logistic regression analysis, other

Table 20.7. Type of Early Abuse (Childhood or Adolescence) and Characteristics of Early Abuse

	Physical Abuse *N*(%)	Sexual Abuse *N*(%)
Early Onset	92 (50)***	96 (50)***
Ultradian Cycling	52 (41)**	43 (34)
Increased Severity of Mania	116 (63)***	106 (56)**
Increased Severity of Depression	119 (64)	123 (64)**
Attempted Suicide	90 (49)***	90 (48)***

*** $p < .001$
** $p < .01$
* $p < .05$

variables remain significant as well. Physical abuse included self-reports of a pattern of increasing severity of mania and a family history of bipolar disorder, alcoholism, drug abuse, or other psychiatric illnesses. Sexual abuse included self-reports of an increased incidence of attempted suicide (45%) and a family history of drug abuse and other psychiatric illnesses.

There was also an increase in the number of clinician-rated Axis I lifetime comorbidities (2.0) in those experiencing these early traumatic life experiences versus those without these early stressors (1.3, $p < 0.002$). Physical abuse was associated with a significant increase in anxiety disorder, drug abuse, alcoholism, and a diagnosis of PTSD. Sexual abuse was selectively associated with a lifetime history of drug abuse. Those with early stressor history also had greater numbers of Axis II comorbidities. Physical abuse had a strong association with increased presence of cluster A disorders (i.e., the odd, eccentric) including paranoid, schizoid, and schizotypical disorders. Sexual abuse was most strongly associated with the presence of cluster B disorders (i.e., dramatic, emotional, including histrionic, narcissistic, borderline, and antisocial disorders).

Moreover, in addition to the retrospective self-reported illness variables related to earlier onset and greater severity in the unfolding of bipolar illness (mania and suicidality), in the prospective year of clinician ratings we observed that those with a history of physical and/or sexual abuse in childhood and adolescence were more ill than those without such a history (Leverich et al., 2002). This was revealed in both an increased percentage of time well measured on the LCM as well as on the increased levels of depression severity measured on the Inventory of Depressive Symptomatology (IDS) (Rush et al., 1986, 1996).

CLINICAL APPROACHES TO BIPOLAR ILLNESS AND ITS PREVENTION

Caveats

The causal relationships in the data described above are not easily discerned or readily disentangled. Although it is highly plausible to first think that these early life experiences could lead to altered neurochemistry through some of the mechanisms described in the previous preclinical sections, and thus change the likelihood and severity of bipolar symptom development and evolution, it is also possible that traits associated with increased severity of later illness could evoke increased physical or sexual abuse. Lastly, it is possible that another or third variable, such as genetic loading, could determine both the more severe pattern of illness and the tendency for increased physical or sexual abuse (either evoked or directly related to parental illness), rather than any direct causal relationship between early abuse and more severe course of illness characteristics.

Notwithstanding these causal ambiguities, the strong relationships suggest the importance of attempts at earlier intervention. Those who were physically or sexually abused had a longer period of time from first affective symptoms to first treatment (13 ± 11 years) than those who were not abused (8 ± 9 years; $p = .0003$). Even eight years in the nonabused group is far too long; the average treatment delay in many populations is about ten years, including the Stanley Network (Suppes et al., 2001), the surveyed members of the National DMDA (Lish, Dime-Meenan, Whybrow, Price, & Hirschfeld, 1994), or other clinical research cohorts (Egeland, Hostetter, Pauls, & Sussex, 2000).

Early Intervention

Thus, there is a great need for earlier recognition and initiation of treatment in patients with bipolar illness in general. It would appear even more critical for those at high risk for more severe illness progression and negative prospective outcomes based on high genetic loading or the occurrence of early stressors. Yet it is just these adolescents and adults who are likely to have the longest delays in beginning treatment.

In addition to helping prevent serious affective dysfunction, earlier intervention may help a child avoid the variety of Axis I (McElroy et al., 2001), Axis II (Leverich et al., 2000), and medical comorbidities that are associated with these early stressful life experiences. In particular, the increased rate of substance abuse is already a problem in general in bipolar illness (Regier et al., 1990), and now we have found there is an additional greater risk for those with these earlier adverse life experiences. Forty-eight percent of those with a history of sexual abuse versus 19 percent without ($p = 0.00025$) have a lifetime diagnosis of drug abuse in the Stanley Foundation Bipolar Network; 40 percent of those with a history of physical abuse compared with 22 percent without have a history of drug abuse, and 52 percent versus 31 percent have a history of alcohol abuse ($p = 0.025$) (Leverich et al., 2000).

Thus, it would appear prudent to recommend primary substance abuse prevention techniques in the child and adolescent with bipolar illness, particularly in the presence of a history of physical and/or sexual abuse in childhood and/or adolescence. One can only wonder about the potential parallels of these vulnerabilities to the findings of increased alcohol and cocaine self-administration in the adult rodents that had previously experienced repeated maternal separation as pups (Meaney et al., 2002; Huot et al., 2002).

The association between stressful episodes and precipitation of the first episode of illness, as well as the current episode of illness in those with a history of early physical or sexual abuse compared with those without, also provides an important area for pharmacotherapeutic and psychotherapeutic intervention. To the extent that these individuals are particularly vulnerable to stressor precipitation of episodes and/or at increased risk of exposure to more stressors in general (Table 20.8), as our data would suggest ($p < 0.0001$ for physical abuse and $p < 0.001$ for first sexual abuse [Leverich et al., 2002]), dealing with this likelihood on a direct basis with appropriate cognitive, behavioral, interpersonal, or other focused psychotherapeutic techniques, as well as pharmacotherapeutics, may be of great assistance in raising the threshold for episode precipitation. Putting coping strategies and alternative perspectives into place that would enable the individual to be more comfortable in the face of stressor occurrence may be particularly valuable. The great potential for therapeutic benefit of these types of interventions are delineated in Cicchetti, Rogosch, and Toth (2000) and Luthar, Cicchetti, and Becker (2000).

Opposite Effects of Stress and Psychotrophic Drugs on Gene Expression and Neurogenesis

New data suggest additional theoretical rationales for psychotherapy besides providing additional psychosocial support based on the general therapeutic relationship and specialized techniques employed. To the extent that therapy and the development of

Table 20.8. Incidence of Stressful Life Events prior to First Episode and Most Recent Episode in Patients with or without a History of Early Abuse (Data from Leverich et al., 2002)

	Prior to First Episode	Prior to Most Recent Episode
History of Early Physical Abuse		
Absent	2.5	2.8
	]***	]***
Present	4.2	4.8
History of Early Sexual Abuse		
Absent	2.5	2.8
	]***	]***
Present	3.9	4.4

*** = $p < 0.001$

coping strategies can lessen the impact of stressors, they could ultimately lessen the effects of stressors on gene expression. Although this remains only a theoretical possibility, it has been demonstrated that antidepressant compounds have a variety of effects on neurotrophic factor gene expression that are opposite to those of stress (Duman, 1998; Smith et al., 1995). For example, stress depletes BDNF in the hippocampus while chronic antidepressant treatment increases BDNF in this area. Moreover, both Smith and colleagues in our laboratory (Smith et al., 1995) and Duman (1998) have demonstrated that pretreatment with antidepressants may block some or all of the associated effects of stress on neurotrophic factor gene expression. Most recently, this paradigm has been extended by Gould and Tanapat (1999) and others, indicating that the antidepressants (and lithium) increase neurogenesis even in the adult animal, whereas stressors produce the opposite effect (Table 20.9).

Table 20.9. Impact of Stress and Psychotropic Drugs on Gene Expression and Brain Structure

	STRESS	Gluco-corticoids	LITHIUM	VPA	TCAs
Transcription Factor CREB	↓	↓	↑		↑
Neurotrophic Factor BDNF	↓	↓	↑		↑
Neuroprotective Factor BCL-2 (Anti-Apoptotic)			↑↑	↑	
Neurite Sprouting (in vitro)		↓	↑		
Neurogenesis (in vivo)	↓	↓	↑		↑
Neuronal Viability (NAA by MRS in humans)			↑		
Increased Grey Matter (in humans)			↑		

Based on studies of Smith et al., 1995; Duman, 1998; Chen and Chuang, 1999; Gould and Tanapat, 1999; Moore et al., 2000a,b; Chuang et al., 2002

Lithium as a Neuroprotectant

Significant effects are not unique to the antidepressant substances because some mood stabilizers also appear to have neurotrophic and neuroprotective properties. Chuang and his collaborators reported neuroprotective effects of lithium in a variety of cell culture systems (Nonaka, Katsube, & Chuang, 1998) and then went on to demonstrate that this occurred *in vivo* as well. Chronic treatment with lithium in rats subjected to ligation of the middle cerebral artery (Nonaka & Chuang, 1998) reduced the size of ischemic infarct by approximately 50 percent. In addition, they observed marked neuroprotective effects of lithium in an animal model of Huntington's disease involving the intrastriatal administration of the neurotoxic compound quinolinic acid (Wei et al., 2001). For example, lithium increases the expression of BDNF and Bcl2 mRNA (two neuroprotective factors), whereas it decreases the mRNA levels of Bax and p53 (two proteins that promote cell death or apoptosis) (Chen & Chuang, 1999; Chen et al., 1999). Lithium increases neurite sprouting in culture and in humans also increases a marker of neural integrity in levels of N-acetyl aspartate (NAA) measured by MRS (Moore et al., 2000b). Taken together, these converging *in vitro* and *in vivo* data in animals and humans suggest the possibility that lithium's neuroprotective properties could be important to its therapeutic effects, although this remains to be more directly demonstrated.

Potential Liabilities of Lithium Discontinuation

These preclinical data are of great interest in relation to the clinical findings that lithium not only helps to bring the markedly elevated suicide rate in the unipolar and bipolar affective disorders back toward normal (Baldessarini, Tondo, & Hennen, 1999; Tondo et al., 1998), but also normalizes the mortality rate from associated medical conditions in patients with primary affective disorders (Ahrens et al., 1995; Coppen et al., 1991). These data raise the possibility that the antistroke and neuroprotective effects of lithium observed in animal models could play a role in the normalization of medical mortality in patients who remain on long-term lithium prophylaxis. These observations provide other secondary reasons for continuing lithium pharmacotherapy, even in the absence of a complete clinical response. New meta-analytic data from Baldessarini, Tondo, and Hennen (1999) have indicated that there is a twenty-fold increased risk of suicide in those individuals who discontinue lithium in the first year, compared with those who remain on lithium treatment.

Thus, there would appear to be a variety of potential liabilities of lithium discontinuation, including: (1) increasing the likelihood of a new episode of mania and depression and its associated morbidity; (2) provoking a serious episode requiring rehospitalization; (3) destabilizing the illness for the long term; and (4) contributing to the lethality of the illness from death by suicide. As to point 3, there are increasing data suggesting that a small percentage of patients who are doing well on lithium, but decide to discontinue their medicine and experience a relapse, will not have as robust a response when they resume treatment as they previously had (Post, Ketter, Speer, Leverich, & Weiss, 2000; Post, Leverich, Altshuler, & Mikalauskas, 1992; Post, Leverich, Pazzaglia, Mikalauskas, & Denicoff, 1993). Even in the study of Tondo et al. (1997), reporting no difference in episodes on lithium prior to and after the lithium discontinuation, a significantly higher dose of neuroleptics was required after the period off lithium.

Table 20.10. Prevalence of Lithium Discontinuation-Induced Refractoriness

Study	Length of Lithium Trial (years)	Induced Refractoriness		Patients	Notes
Post et al., 1992, 1993	6–15	9/66	13.6%	All Refractory	Depression or mania
Bauer, 1994	12	1/1	–	–	Single case
Koukopoulos et al., 1995	8.8	13/145	9%	All	Depression or mania
Maj et al., 1995	5.9	10/54	18.5%	Responders	Depression or mania; D/C refractory patients had longer lithium trials
Berghofer and Muller-Oerlinghausen, 1996	5	1/24	4.2%	All	2 initial nonresponders responded in second trial
		1/10	10.0%	Responders	Depression or mania
Tondo et al., 1997	4.6	16/86	18.6%	All	11 initial nonresponders responded in second trial; depression or mania
Coryell et al., 1998	?	1/28	3.6%	Responders	Mania
Overall incidence		39/321	12.1%	All Patients	
		12/92	13.0%	Responders Only	

In our studies and those of a variety of others, 5–15 percent of patients appeared to experience lithium discontinuation-induced refractoriness, in which even the reinstitution of lithium at higher doses than previously needed was without adequate effect (Table 20.10). Coryell et al (1998) reported little evidence for this phenomenon in their report. However, because their study: (a) was under-powered to observe this effect on a statistically reliable basis; (b) used episode criteria that were not optimal; and (c) chose subjects who were not necessarily well-established, long-term lithium responders, one wonders about the strength of the conclusions that can be drawn from that study. Moreover, (d) one of the patients reported in their study failed to re-respond, yielding a refractoriness rate of 3.6 percent even in this negative study (see Table 20.10).

Such a phenomenon of lithium discontinuation-induced refractoriness, when it does occur in an individual, can be particularly devastating, as it was for the patient illustrated in our report (Post, Leverich, Altshuler, & Mikalauskas, 1992). This patient had been completely asymptomatic for eight years during lithium monotherapy treatment, after it was initiated for a series of incapacitating depressions of 2–3 months duration as well as interposed hypomanic episodes. Ten years after restarting her lithium and subsequently adding or substituting a vast array of other treatments, she still continues to be highly symptomatic from her bipolar II illness.

Thus, in addition to the four clinical reasons enumerated above, there is a potential fifth reason for not stopping lithium prophylaxis – to the extent that lithium's neuroprotective effects are related to its mechanisms of action in the affective disorders, one could be losing such a potential long-term protective effect. Could lithium

be preventing not only episodes, but also the neural and glial loss associated with the illness, as described below? Even if lithium's neuroprotective effects were mediated separately from its therapeutic actions in bipolar affective disorders, such discontinuation of lithium might also put the patient at greater medical risk from stroke (separate from the risk of relapse, rehospitalization, refractoriness, or suicide).

Affective Illness and Brain Structure

Alterations in neurochemical content (Knable, Torrey, Webster, & Bartko, 2001; Xing et al., 2002) and in the structure of the brain (Ketter, George, Kimbrell, Benson, & Post, 1997; Soares & Mann, 1997) are increasingly being documented in the affective disorders, and therefore the potential effects of antidepressants and mood stabilizers on neurotrophic factors, neurogenesis, glial survival, and neuronal structure take on added interest. A series of studies have suggested deficient size, area, and number of glia or neurons in areas of the brain including ventral (Drevets, Ongur, & Price, 1998) and dorsal aspects of the anterior cingulate gyrus (Rajkowska et al., 1999) and mixed reports on the size of the hippocampus. Two studies (but not Pearlson et al., 1997) report increased size of the amygdala (Altshuler et al., 2000; Strakowski et al., 1999), one in proportion to the number of hospitalizations for mania (Altshuler et al., 2000).

Thus, we return to the preclinical data reviewed above indicating that experiential effects on gene expression could induce long-lasting changes in behavioral and neurochemical set points, as well as in synaptic and neuroanatomical structure and altered numbers of neuronal and glial cells. The impact of life events on brain biochemistry and microstructure could likewise be of either pathological or adaptive importance for the clinical course of the affective disorders as well. The plasticity of the brain is extraordinary and ongoing throughout one's life. With better understanding of not only the genetic underpinning of vulnerability to affective disorders, but also their interaction with crucial life events and recurrent episodes of illness, we should ultimately be able to design more rational approaches to psychological and pharmacological interventions at the appropriate opportunities.

The Possibility of Primary Prevention

As clinical and genetic markers of high vulnerability become better recognized, perhaps a role for primary prevention in those at highest risk (even before full expression of the illness) should begin to be considered. To the extent that early intervention (such as at first symptoms yielding dysfunction) helps prevent the development of more full-blown recurrent affective disorders and their progression toward treatment-refractoriness even in only a subgroup of patients, an important impact on many lives would be achieved.

Although most of the links between preclinical models and pathophysiological mechanisms in the affective disorders discussed here remain at the level of hypothesis generation and require more direct examination and testing, their ability to engender appropriate clinical questions and conceptualize the longer time domains of vulnerability (including over the entire lifetime of an individual) gives them value, even beyond their direct predictive validity. We hope that this speculative discussion will foster a wide range of questions and concepts that ultimately will lead to earlier, more focused, and rational interventions in the recurrent affective disorders. Perhaps with such early

intervention, the magnitude of the problem affective disorders pose for individuals and society – at both the level of immense suffering and in the billions of dollars they cost each year – can be very substantially lessened.

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APPENDIX 3

Stress Impairs α_{1A} Adrenoceptor-Mediated Noradrenergic Facilitation of GABAergic Transmission in the Basolateral Amygdala

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Intense or chronic stress can produce pathophysiological alterations in the systems involved in the stress response. The amygdala is a key component of the brain's neuronal network that processes and assigns emotional value to life's experiences, consolidates the memory of emotionally significant events, and organizes the behavioral response to these events. Clinical evidence indicates that certain stress-related affective disorders are associated with changes in the amygdala's excitability, implicating a possible dysfunction of the GABAergic system. An important modulator of the GABAergic synaptic transmission, and one that is also central to the stress response is norepinephrine (NE). In the present study, we examined the hypothesis that stress impairs the noradrenergic modulation of GABAergic transmission in the basolateral amygdala (BLA). In control rats, NE (10 μM) facilitated spontaneous, evoked, and miniature IPSCs in the presence of β and α_2 adrenoceptor antagonists. The effects of NE were not blocked by α_{1D} and α_{1B} adrenoceptor antagonists, and were mimicked by the α_{1A} agonist, A61603 (1 μM). In restrain/tail-shock stressed rats, NE or A61603 had no significant effects on GABAergic transmission. Thus, in the BLA, NE acting via presynaptic α_{1A} adrenoceptors facilitates GABAergic inhibition, and this effect is severely impaired by stress. This is the first direct evidence of stress-induced impairment in the modulation of GABAergic synaptic transmission. The present findings provide an insight into possible mechanisms underlying the antiepileptogenic effects of NE in temporal lobe epilepsy, the hyperexcitability and hyper-responsiveness of the amygdala in certain stress-related affective disorders, and the stress-induced exacerbation of seizure activity in epileptic patients.

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INTRODUCTION

Many components of the biological response to emotional stressors are of vital importance in enabling the individual to cope with stress. However, it is well known that excessive or repeated stress can have detrimental effects on health that are often associated with functional alterations in the systems involved in the stress response (Vermetten and Bremner, 2002; Vanitallie, 2002; McEwen, 2002; Pawlak *et al*, 2003). The amygdala is a key component of the brain's neuronal network that determines the emotional significance of external events (LeDoux, 1992; Davis, 1994; Breiter *et al*, 1996; Schneider *et al*, 1997; LaBar *et al*, 1998; Buchel *et al*, 1998; Whalen *et al*, 1998; Baird *et al*, 1999; Davidson *et al*, 1999; Davidson and Slagter, 2000; Buchel and Dolan, 2000). Via efferent pathways to the hypothalamus, the amygdala can also trigger the neuroendocrine cascades that are part of the stress response (Habib *et al*, 2001; Pitkänen, 2000; Davis, 1992) and via reciprocal connections with the cerebral cortex and limbic structures, it modulates the orchestration of the behavioral response (Goldstein *et al*, 1996; Pitkanen *et al*, 2000). Therefore, understanding the changes in the amygdala's physiology and function induced by stress is critical in understanding the pathophysiology of stress, and may aid the development of new therapeutic strategies for the prevention and treatment of stress-related, affective disorders.

Different lines of evidence point to the possibility that the function of the GABAergic system may be impaired by stress. First, in a number of brain regions, benzodiazepine receptor binding is altered by stress (Lippa *et al*, 1978; Medina *et al*, 1983; Miller *et al*, 1987; Weizman *et al*, 1989; Bremner *et al*, 2000). Second, in certain stress-related psychiatric disorders, the amygdala exhibits higher than normal levels of basal activity (Abercrombie *et al*, 1998; Drevets, 1999), or exaggerated responses to fearful stimuli (Rauch *et al*, 2000; Villarreal and King, 2001). Since the GABAergic system is a primary regulator of neuronal excitability, pathophysiological changes in GABAergic transmission may underlie the amygdala's hyper-responsiveness and hyperexcitability in these emotional disorders.

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Third, many psychotropic drugs that are effective in the treatment of emotional disorders target or influence GABAergic transmission. Fourth, stress exacerbates the frequency of seizures in epileptic patients (Temkin and Davis, 1984; Frucht *et al*, 2000). However, there is no direct evidence, so far, for stress-induced impairment in GABAergic synaptic transmission.

One of the modulators of GABA release is norepinephrine (NE), which is also central to the stress response. During stress, there is a dramatic increase in noradrenergic activity following the peripheral release of epinephrine from the adrenal glands, and the central release of NE, predominantly from the locus ceruleus (Stanford, 1995; Bremner *et al*, 1996). The amygdala receives dense noradrenergic afferents from the locus ceruleus (Pitkänen, 2000), as well as from other brain regions such as the nucleus of the solitary tract (Pitkänen, 2000; Clayton and Williams, 2000; Williams *et al*, 2000). During stress, there is a strong enhancement of NE release in the amygdala (Galvez *et al*, 1996; Stanford, 1995; Quirarte *et al*, 1998; Tanaka *et al*, 2000). The short- and long-term consequences of stress-induced excessive NE release on amygdala's physiology are unknown.

NE modulates GABAergic inhibition primarily via the α_1 subtype of adrenergic receptors (Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Kawaguchi and Shindou, 1998). There is evidence suggesting that α_1 adrenoceptors are affected by stress. Thus, chronic stress, in rats, reduces the expression of these receptors in the hypothalamus and brain stem (Miyahara *et al*, 1999). α_1 adrenoceptor binding is also reduced in depressed patients (Crow *et al*, 1984; Gross-Isseroff *et al*, 1990), and blockade of these receptors in rats increases depressive behavior (Stone and Quartermain, 1999). The physiological implications of stress-induced reduction in α_1 adrenoceptor activity are not known.

In the present study, we investigated whether NE modulates GABAergic transmission in the basolateral nucleus of the amygdala (BLA), and if so, whether the noradrenergic modulation of the GABAergic transmission is altered by exposure to stress. We studied the BLA because this amygdala region is heavily involved in the processing of emotional experiences, as it receives both direct and indirect thalamic and cortical inputs and is extensively interconnected with the prefrontal/frontal cortex and the hippocampus (Pitkänen, 2000). Furthermore, it appears that the BLA selectively (among the different amygdala nuclei) modulates the consolidation of emotional memories (Cahill and McGaugh, 1998; Ferry *et al*, 1999). Our results show that NE facilitates spontaneous, evoked, and action potential-independent, quantal GABA release in the BLA via the α_{1A} subtype of adrenergic receptors, and that exposure to stress severely impairs this α_1 adrenoceptor-mediated facilitation of GABA release.

METHODS

Animals and Stress Protocol

All animal experiments were performed in accordance with our institutional guidelines after obtaining the approval of the Institutional Animal Care and Use Committee (IACUC). Male, Sprague–Dawley rat pups were received with their mother at postnatal day (PND) 17, and housed in a climate-controlled environment on a 12 h light/dark cycle (lights on at 0700). On PND 21, the rats were weaned, assigned numbers, and randomly divided into control and stressed groups. They were housed individually, with food and water supplied *ad libitum*. The 'stressed group' was exposed to stress on PND 22, 23, and 24. The rats were killed and brain slices were prepared on PND 24 and 25. The experiments were performed in a blind manner. The investigators did not know whether they used a control or a stressed rat until the data were analyzed.

Stress exposure consisted of a 2-h per day session of immobilization and tail-shocks, for 3 consecutives days. The animals were stressed in the morning (between 0800 and 1200). They were restrained in a plexiglas tube, and 40 electric shocks (2 mA, 3 s duration) were applied at varying intervals (140–180 s). This stress protocol was adapted from the 'learned helplessness' paradigm in which animals undergo an aversive experience under conditions in which they cannot perform any adaptive response (Seligman and Maier, 1967; Seligman and Beagley, 1975). We stressed the rats for 3 consecutive days because it has been previously demonstrated that repeated stress sessions for 3 days is more effective than a single stress session in producing physiological and behavioral abnormalities, such as elevations in the basal plasma corticosterone levels, exaggerated acoustic startle responses, and reduced body weight (Servatius *et al*, 1995; Ottenweller *et al*, 1989). More stress sessions, beyond the 3 days, do not appear to produce greater physiological and behavioral changes (Servatius *et al*, 1995; Ottenweller *et al*, 1989).

Slice Preparation

Experimental procedures. The amygdala slice preparation has been described previously (Li *et al*, 2001). Briefly, the rats were anesthetized with halothane and then decapitated. The brain was rapidly removed and placed in an ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 2.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 25 $NaHCO_3$, 1.25 NaH_2PO_4, and 11 glucose, bubbled with 95% O_2/5% CO_2. A block containing the amygdala region was prepared by rostral and caudal coronal cuts, and coronal slices, 400 µm thick, were cut using a Vibratome (series 1000, Technical Products International, St Louis, Missouri). Slices were kept in a holding chamber containing oxygenated ACSF at room temperature, and experiments started $\geqslant$1 h after slice preparation.

Electrophysiology

For whole-cell recordings, slices were transferred to a submersion-type recording chamber where they were continuously perfused with oxygenated ACSF at a rate of 4 ml/min. All experiments were carried out at 32°C. Tight-seal (>1 GΩ) whole-cell recordings were obtained from the cell body of neurons in the BLA region. Patch electrodes were fabricated from borosilicate glass and had a resistance of 1.5–5.0 MΩ when filled with a solution containing (in mM) Cs-gluconate, 135; $MgCl_2$, 10; $CaCl_2$, 0.1; EGTA, 1; HEPES, 10; QX-314, 20; NaATP, 2; Na_3GTP, 0.2 and Lucifer yellow, 0.4% (pH 7.3, 285–290 mOsm). Neurons were

visualized with an upright microscope (Nikon Eclipse E600fn) using the Nomarski-type differential interference optics through a ×60 water immersion objective. Neurons with a pyramidal appearance were selected for recordings. During whole-cell recordings, neurons were filled passively with 0.4% Lucifer yellow (Molecular Probes, Eugene, Oregon) for *post hoc* morphological identification, as described previously (Braga *et al*, 2003). The fluorescence image of the dye-filled neurons was captured by a Leica DM RXA fluorescence microscope equipped with an SPOT2 digital camera and a laser scanning confocal microscope (Bio RAD, MRC-600). Neurons were voltage clamped using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Inhibitory postsynaptic currents (IPSCs) were pharmacologically isolated and recorded at a holding potential of −70 mV. Synaptic responses were evoked with sharpened tungsten bipolar stimulating electrodes (2 μm diameter, World Precision Instruments, Sarasota, Florida) placed in the BLA, 50–100 μm from the recording electrode. Stimulation was applied, at 0.1 Hz, using a photoelectric stimulus isolation unit having a constant current output (PSIU6, Grass Instrument Co., W. Warwick, RI). Access resistance (8–26 MΩ) was regularly monitored during recordings, and cells were rejected if it changed by more than 15% during the experiment. The signals were filtered at 2 kHz, digitized (Digidata 1322A, Axon Instruments, Inc.), and stored on a computer using the pCLAMP8 software (Axon Instruments, Inc.). The peak amplitude, 10–90% rise time, and the decay time constant of IPSCs were analyzed off-line using pCLAMP8 software (Axon Instruments) and the Mini Analysis Program (Synaptosoft, Inc., Leonia, NJ). Miniature IPSCs (mIPSCs) were analyzed off-line using the Mini Analysis Program (Synaptosoft, Inc., Leonia, NJ), and detected by manually setting the threshold for each mIPSC after visual inspection.

For field potential recordings, slices were transferred to an interface-type recording chamber maintained at 32°C, where they were perfused with ACSF at 0.7–1 ml/min. Field potentials were recorded in the BLA, while stimulation was applied to the external capsule, at 0.05 Hz (Aroniadou-Anderjaska *et al*, 2001). Recording glass pipettes were filled with 2 N NaCl (2–5 MΩ). Bipolar stimulating electrodes were constructed from twisted, stainless-steel wires, 50 μm in diameter. The field potentials were filtered at 1 kHz, and digitized on-line at 5 kHz.

All data are presented as mean ± SEM. For body weight data, sample size *n* refers to the number of rats. For electrophysiological experiments, sample size *n* refers to the number of slices. This corresponds to the number of neurons, in whole-cell recordings, as a single neuron was studied from each slice. From each rat, two slices were used for each type of experiment (whole-cell recordings or field potential recordings). The results were tested for statistical significance using the Student's paired *t*-test.

Drugs

The following drugs were used: D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, Tocris Cookson, Ballwin, Missouri), an NMDA receptor antagonist; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris Cookson, Ballwin, Missouri), a potent AMPA/kainate receptor antagonist; (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid (SCH50911, Tocris Cookson, Ballwin, Missouri), a $GABA_B$ receptor antagonist; bicuculline methiodide (Sigma), a $GABA_A$ receptor antagonist; tetrodotoxin (TTX, Sigma), a sodium channel blocker; DL-propranolol (Sigma), a β adrenoceptor antagonist; (1-[4-amono-6,7-dimethoxy-2-quinazolinyl]-4-[2-furanylcarbonyl]-piperazine hydrochloride (prazosin, Sigma), an α_1 adrenoceptor antagonist; yohimbine hydrochloride (Sigma), an α_2 adrenoceptor antagonist; *N*-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro-naphthalen-1-yl]methanesulfonamide hydrobromide (A61603, Tocris Cookson, Ballwin, Missouri), a selective α_{1A} agonist (Knepper *et al*, 1995); chloroethylclonidine (CEC, Sigma), an irreversible antagonist that blocks both α_{1B} and α_{1D} adrenoceptors (Xiao and Jeffries, 1998); 8-[2-[4-(2-methoxyphenyl)-1-piperazinil]ethyl]-8-azaspiro[4,5]-decane-7,9-dione dihydrochloride (BMY 7378, Tocris Cookson, Ballwin, Missouri), a selective antagonist of α_{1D} adrenoceptors (Deng *et al*, 1996; Saussy Jr *et al*, 1996); 2-(2,6-dimethoxyphenoxyethy)aminomethyl-1,4-benzodioxane hydrochloride (WB4101, Tocris Cookson, Ballwin, Missouri), a selective antagonist of the α_{1A} adrenoceptor (Zhong and Minneman, 1999).

RESULTS

The body weight of the control and stressed rats was measured daily between 1400 and 1500. The control rats were 44.5 ± 1.5 g ($n=24$) on PND 21 and 58.8 ± 1.9 g ($n=24$) on PND 24 (Figure 1). The body weight of the stressed group was 44.2 ± 1.8 g ($n=23$) before the first stress session on PND 21, and 51.0 ± 2.3 g ($n=20$) after the last stress session, on PND 24. The difference in body weight between stressed and control rats was statistically significant after the second day of stress ($p<0.01$). Stressed rats that were not used for electrophysiological experiments continued to display reduced body weight gain for as long as body weight was monitored (up to 10 days after stressor cessation, data not shown).

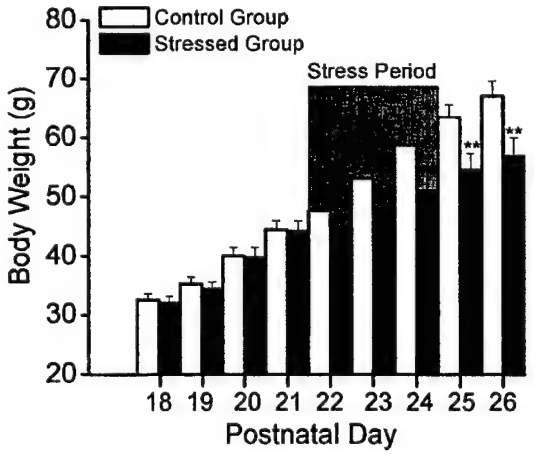


Figure 1 Restrain/tail-shock stress reduces body weight gain. Exposure to stress on PNDs 22, 23, and 24 reduced body weight gain. The body weight difference between control and stressed rats was statistically significant after the first day of stress (**$p<0.01$). Data on PND 26 are from rats that were not used for electrophysiological experiments. Sample sizes range from 12 (PND 26) to 24 rats.

Stress Blocks Noradrenergic Facilitation of GABAergic Synaptic Transmission

Noradrenergic modulation of spontaneous IPSCs (sIPSCs). To investigate whether NE modulates GABAergic transmission in the BLA, and whether stress alters this modulation, we first examined the effects of NE on action-potential dependent, sIPSCs recorded from BLA pyramidal neurons, in control and stressed rats. sIPSCs were recorded at a holding potential of −70 mV, and in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM) to block NMDA, AMPA/kainate, and β and α_2 receptors, respectively. In control rats, the mean frequency of sIPSCs recorded from the soma of BLA pyramidal neurons was 3.1 ± 1.6 Hz ($n=21$). Bath application of bicuculline (10 μM) eliminated sIPSCs, confirming that they were mediated by $GABA_A$ receptors. NE at 1, 10, and 100 μM produced a dose-dependent enhancement in the frequency and amplitude of sIPSCs (Figure 2). At 100 μM of NE, the enhancement of sIPSCs was too high to be quantified precisely. The 10 μM concentration appeared to be close to the EC_{50}, and therefore it was used in subsequent experiments. After the application of 10 μM NE, the mean frequency of sIPSCs was increased to 984.39 ± 148.2% of the control values ($n=21$, $p<0.01$; Figure 3a). The amplitude of sIPSCs was increased to 144.0 ± 12.8% of the control values ($n=21$, $p<0.05$; Figure 3a). These effects persisted throughout the application of NE and were completely reversed after removal of the agonist. The effects of NE were not accompanied by any significant change in the rise time or decay time constant of sIPSCs (Figure 3a), and were blocked by the α_1 adrenoceptor antagonist prazosin (1 μM, Figure 3c), confirming that NE was acting via α_1 adrenergic receptors.

In stressed rats, the mean frequency of sIPSCs was 2.6 ± 2.3 Hz. NE (10 μM) had no significant effect on the frequency or amplitude of sIPSCs. Thus, in the presence of NE (10 μM), the frequency of sIPSCs was 128.9 ± 19.2% and the amplitude was 111.4 ± 10.2% of the control values ($n=19$, Figure 3b). In addition, bath perfusion of NE (10 μM) caused no significant changes in the kinetics of these currents (rise time and decay time constant of sIPSCs; Figure 3b).

To identify the subtype of α_1 adrenoceptors involved in the effects of NE on control rats, we first applied NE (10 μM) in the additional presence of CEC (10 μM) and BMY 7378 (300 nM) to block α_{1B} and α_{1D} adrenoceptors. There was no significant attenuation of the effects of NE in the presence of these antagonists (Figure 4). Thus, NE increased the frequency of sIPSCs from 2.8 ± 2.4 to 27.1 ± 7.9 Hz ($p<0.01$, $n=6$; Figure 4), and the amplitude of sIPSCs to 154 ± 11.3% of the control values ($p<0.05$, $n=6$; Figure 4).

Next, we examined the effects of the specific α_{1A} adrenoceptor agonist A61603. In control rats, A61603 (1 μM) increased the frequency and amplitude of sIPSC to 1034 ± 158.6 and 162 ± 14.2% of the control values, respectively ($p<0.01$, $n=16$; Figure 5a). There were no effects on the rise time or the decay time constant of sIPSCs (Figure 5a). In stressed rats, A61603 had no significant effect (Figure 5b). Thus, in the presence of 1 μM A61603 the frequency of sIPSCs was 132 ± 21% and the amplitude of sIPSCs was 106 ± 8.8% of the control values ($n=18$, Figure 5b). The effects of A61603 on sIPSCs in control rats were blocked by the selective α_{1A} adrenoceptor antagonist WB4101 (1 μM, Figures 5c and d).

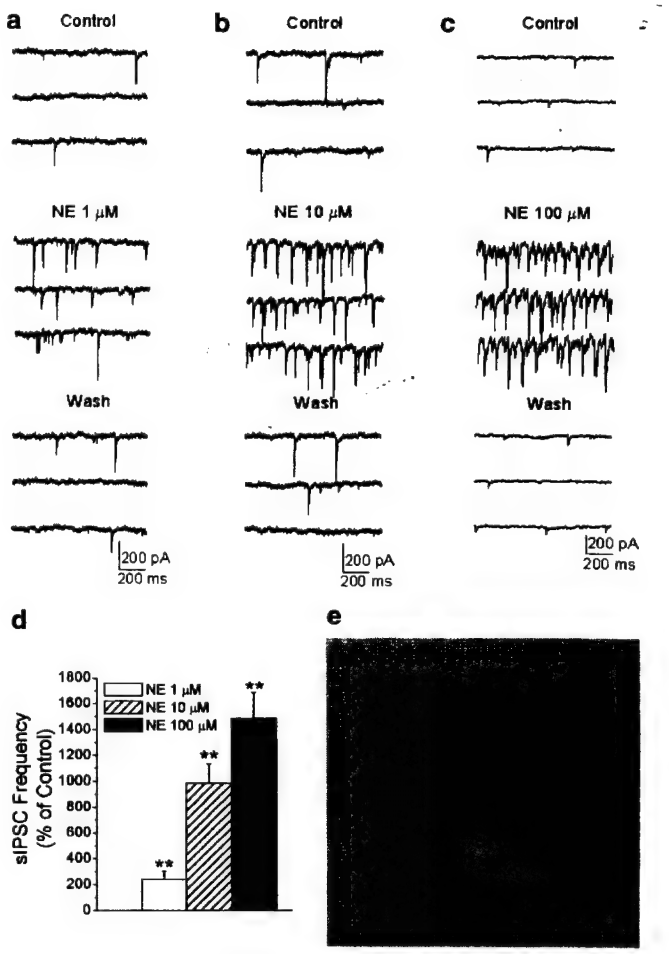
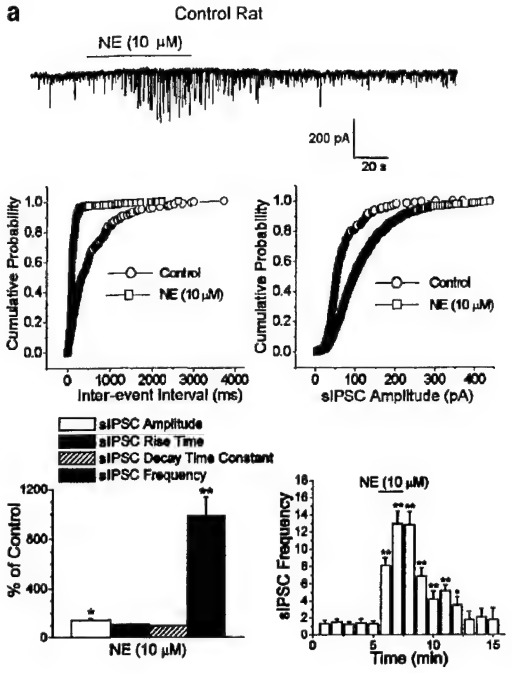
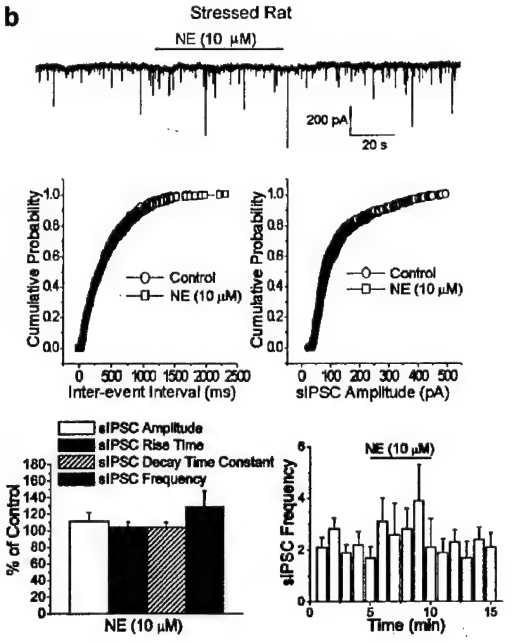


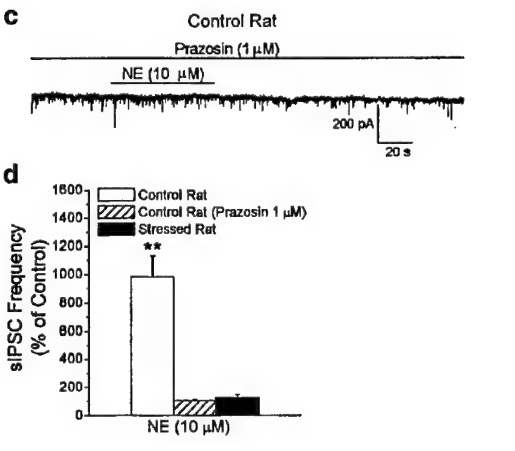
Figure 2 Activation of α_1 adrenoceptors increases tonic inhibition of BLA pyramidal neurons in a dose-dependent manner. (a–c) sIPSCs recorded from three different cells are shown. The holding potential is −70 mV. The medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). The application of 1, 10, and 100 μM NE increased the frequency of sIPSCs in a dose-dependent manner. The bar graph (d) shows group data of the increase of sIPSC frequency ($n=8$ for each concentration of NE, $^{**}p<0.01$). (e) Photomicrograph of pyramidal cell (b) showing the typical morphology of the recorded neurons. The cell has been labeled with Lucifer Yellow. Scale bar, 40 μm.

Taken together, these results suggest that (1) NE, acting via α_{1A} adrenoceptors, enhances tonic inhibition of pyramidal cells in the BLA by inducing a massive increase in action potential-dependent spontaneous release of GABA, and (2) stress impairs this function of NE.

Noradrenergic modulation of evoked IPSCs (eIPSCs). It has been shown previously that NE reduces evoked inhibitory transmission in the hippocampus via α adrenoceptors (Madison and Nicoll, 1988; Doze *et al*, 1991). More recently, in the sensorimotor cortex, it was found that NE actually has a small facilitatory effect on eIPSCs, which is detected when $GABA_B$ receptors are blocked (Bennett *et al*, 1997). To determine the effects of NE on evoked inhibitory







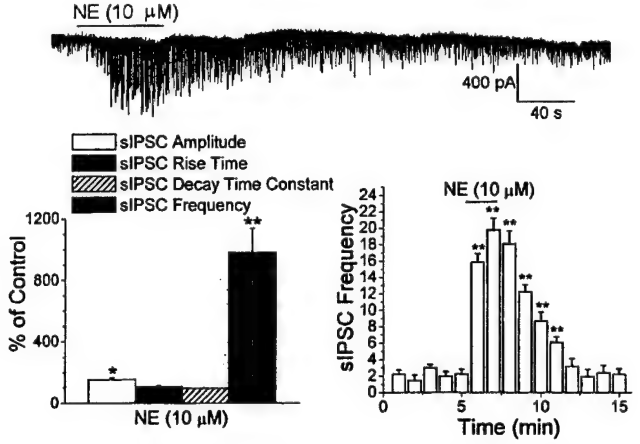
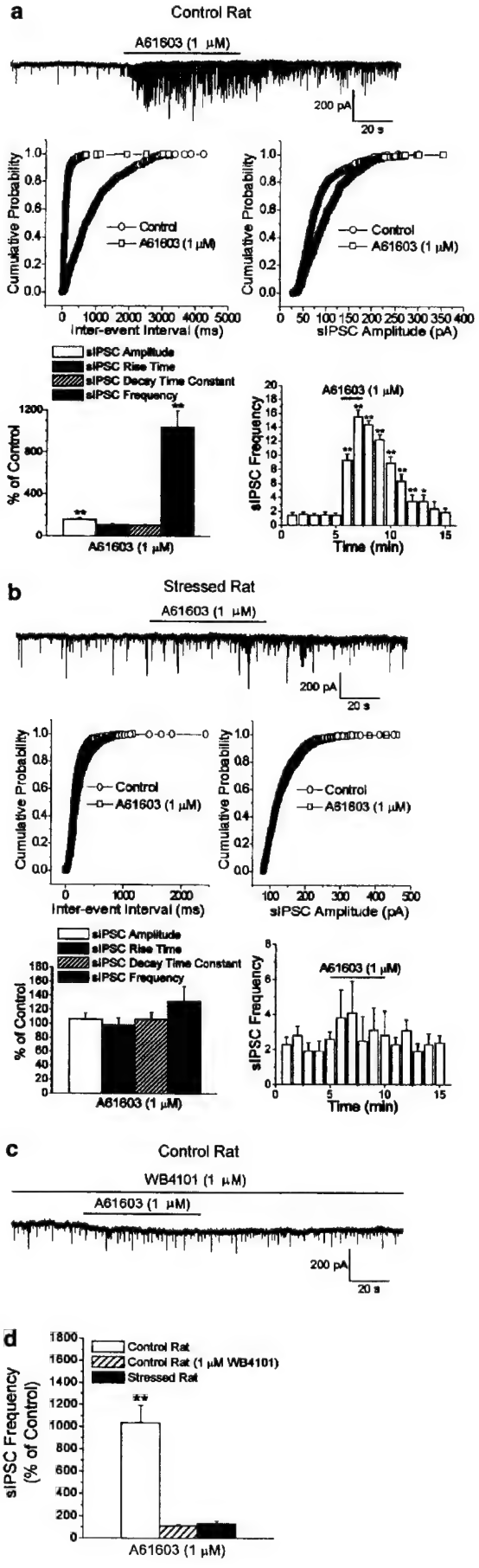


Figure 4 The NE-induced enhancement of sIPSCs is not blocked by α_{1B} and α_{1D} adrenoceptor antagonists. Top trace: sIPSCs recorded from a BLA pyramidal cell of a control rat (holding potential is −70 mV). Bath application of NE (10 μM) in the presence of D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), yohimbine (20 μM), CEC (10 μM), and BMY 7378 (300 nM) reversibly increased the frequency and amplitude of sIPSCs. The bar graph shows pooled data (mean ± SEM) from six neurons. $*p<0.05$, $**p<0.01$.

transmission in the BLA we applied 10 μM NE while recording eIPSCs in control rats. In the absence of a $GABA_B$ receptor antagonist, NE (10 μM) reduced the amplitude of eIPSCs to 48.2 ± 10.3% of the control levels ($p<0.01$, $n=8$; Figure 6). However, in the presence of SCH50911 (20 μM), a specific antagonist of the $GABA_B$ receptors, NE enhanced the amplitude of eIPSCs to 162.4 ± 9.3% of the control, $p<0.01$, $n=10$; Figure 7a) without affecting the rise time and decay time constant of the eIPSCs (Figure 7a). Similar effects were obtained when α_{1A} adrenoceptors were activated by the application of 1 μM A61603 (Figure 7c). Thus, A61603 (1 μM) increased the amplitude of eIPSCs to 159.4 ± 10.7% of the control ($p<0.01$, $n=8$, Figure 7c) without affecting the kinetics of the eIPSCs (Figure 7c). The effects of the drugs were

Figure 3 Activation of α_1 adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (a) Top trace: effects of NE (10 μM) on sIPSCs recorded from a BLA pyramidal cell of a control rat. The holding potential is −70 mV. The medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). Middle graphs: cumulative probability plots of interevent intervals and amplitude of sIPSCs, in control conditions and during NE perfusion (same cell as in the top trace). Bottom graphs: pooled data (mean ± SEM) from 21 neurons. The bar graph on the left shows the NE-induced changes in amplitude, frequency, and kinetics of sIPSCs. The bar graph on the right panel shows the time course of changes in sIPSC frequency during the application of NE. $*p<0.05$, $**p<0.01$. (b) Top trace: sIPSCs recorded from a BLA pyramidal cell of a stressed rat (the holding potential is −70 mV); NE (10 μM) had no significant effect. Middle graphs: cumulative probability plots of interevent intervals and amplitude of sIPSCs in control conditions and during NE perfusion (same cell as in the top trace). Bottom graphs: pooled data (mean ± SEM) from 19 neurons. Effects of NE on the amplitude, kinetics, and frequency of sIPSCs in stressed rats. (c) Prazosin (1 μM) prevented the NE-induced increase of sIPSCs observed in control rats. (d) The bar graph shows the effects of NE on the mean frequency of sIPSCs recorded from control rats (in the absence and in the presence of prazosin), and stressed rats (in the absence of prazosin). $*p<0.05$, $**p<0.01$.



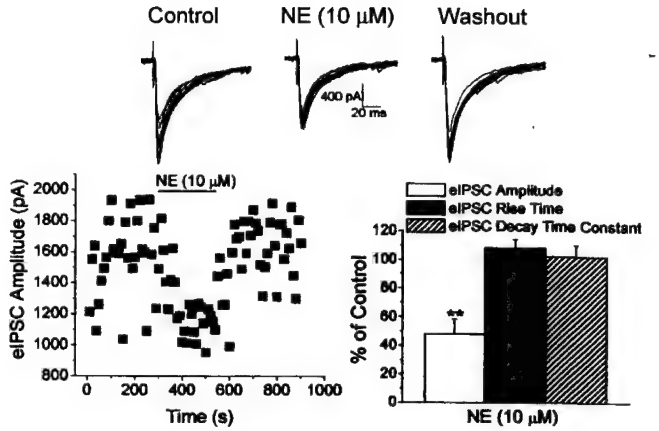


Figure 6 Activation of α_1 adrenoceptors reduces the amplitude of eIPSCs in control rats. Top traces: eIPSCs recorded from a BLA neuron of a control rat. The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). NE reduced the amplitude of eIPSCs with no significant effect on their kinetics. Bottom graphs: the plot shows the time course of the NE effects on the amplitude of eIPSCs (same cell as in top traces). The bar graph shows the relative (% of control) NE-induced changes in amplitude and kinetics of eIPSCs. Pooled data from eight neurons. $^{**}p<0.01$.

reversible. In stressed rats, neither NE nor A61603 had a significant effect on the amplitude, rise time, and decay time constant of eIPSCs (Figure 7b and d). In the presence of NE (10 μM), the eIPSC amplitude was 109 ± 8.2% of the control ($n=11$), and in the presence of A61603, the amplitude of the eIPSCs was 103 ± 7.4 % of the control ($n=10$). These results suggest that (1) NE facilitates evoked the GABAergic transmission via α_{1A} adrenergic receptors, (2) this facilitatory effect is masked due to the activation of presynaptic $GABA_B$ autoreceptors following the NE-induced enhancement of spontaneous GABA release, and (3) stress blocks the facilitatory effect of NE on evoked GABA release.

Noradrenergic modulation of mIPSCs. The enhancement of eIPSCs and action-potential-dependent sIPSCs by NE could be due to a depolarizing effect via the activation of

Figure 5 Activation of α_{1A} adrenoceptors increases tonic inhibition of BLA pyramidal neurons in control rats, but not in stressed rats. (a) Top trace: sIPSCs recorded from a BLA pyramidal cell of a control rat (the holding potential is −70 mV). Bath application of A61603 (1 μM), a specific α_{1A} adrenoreceptor agonist, reversibly increased the frequency and amplitude of sIPSCs. The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). Middle graphs: cumulative probability plots of sIPSC interevent intervals and amplitude in control conditions and during A61603 perfusion (same cell as in the top trace). Bottom graphs: bar graphs show pooled data (mean ± SEM) from 16 neurons. (b) sIPSCs recorded from a BLA pyramidal cell of a stressed rat (the holding potential is −70 mV). Bath application of A61603 (1 μM) caused no significant change in the frequency or amplitude of sIPSCs. Middle graphs: cumulative probability plots of sIPSCs interevent intervals and amplitude in control conditions and during A61603 (1 μM) perfusion (same cell as in the top trace). Bottom graphs: bar graph shows pooled data (mean ± SEM) from 18 neurons. (c) WB4101 (1 μM) prevented the A61603-induced effects observed in control rats. (d) Bar graph shows the effects of A61603 (1 μM) on the mean frequency of sIPSCs recorded from control rats (in the absence and in the presence of WB4101), and stressed rats (in the absence of WB4101). $^{*}p<0.05$, $^{**}p<0.01$.

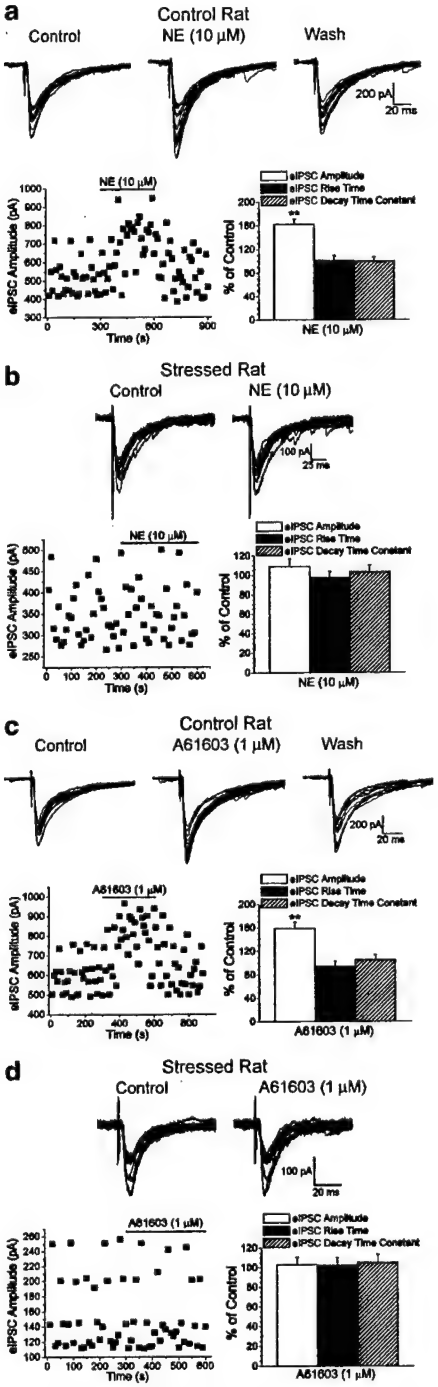


Figure 7 In the presence of a $GABA_B$ receptor antagonist, activation of α_{1A} adrenoceptors increases the amplitude of eIPSCs in control rats, but not in stressed rats. (a) Top traces: eIPSCs recorded from a BLA pyramidal cell of a control rat. In addition to D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM), the slice medium also contains 20 μM SCH50911. NE increased the amplitude of the eIPSCs, without affecting their kinetics. Bottom graphs: the plot shows the time course of the NE effect on eIPSC amplitude (same cell as in the top traces). The bar graph shows the effect of NE on the amplitude and kinetics of eIPSCs. Pooled data from 10 neurons. $^{**}p<0.01$. (b) Data similar to those shown in (a), but from stressed rats. The bar graph shows pooled data from 11 neurons. (c) In control rats, the α_{1A} agonist A61603 produced similar effects to those of NE. Top traces and bottom left plot show data from the same cell. The bar graph shows pooled data from eight BLA neurons. (d) In stressed rats, A61603 had no significant effects on eIPSCs. The bar graph shows pooled data from 10 BLA neurons.

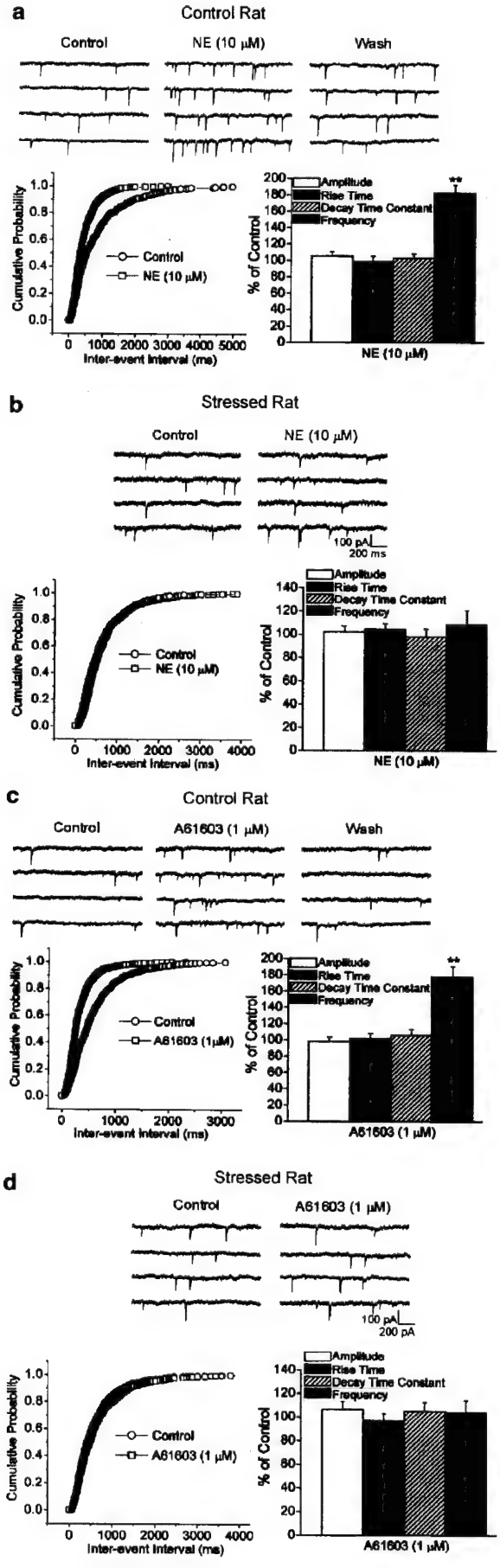
somatodendritic α_{1A} adrenoceptors on GABAergic neurons, and/or due to a direct effect at GABAergic terminals. To determine whether NE modulates GABA release by a direct effect on GABAergic terminals in the BLA, we tested the effects of NE on mIPSCs, which do not depend on the presynaptic invasion of action potentials or Ca^{2+} influx. mIPSCs were recorded in a medium containing D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), yohimbine (20 μM), and TTX (1 μM). In the absence of NE, the frequency of mIPSCs was 0.68 ± 0.32 Hz and their amplitude was 114.0 ± 12 pA ($n=10$). NE (10 μM) increased the frequency of mIPSCs to 182.3 ± 9.6 % of the control levels ($p<0.01$, $n=10$; Figure 8a). The amplitude, rise time, and decay time constant of the mIPSCs were not significantly affected by 10 μM NE (Figure 8a). Similar effects were observed after the application of the α_{1A}-specific agonist A61603 (Figure 8c). A61603 (1 μM) increased the frequency of mIPSCs from 0.71 ± 0.24 to 1.28 ± 0.31 (178 ± 12.4% of the control, $p<0.01$, $n=9$; Figure 8c). The amplitude and kinetics of mIPSCs were not affected by A61603 (Figure 8c).

In stressed rats, neither NE (10 μM) nor A61603 (1 μM) produced a significant effect on mIPSCs frequency, amplitude, or kinetics (Figure 8b and d). Thus, the frequency of mIPSCs was 0.68 ± 0.25 and 0.64 ± 0.34 Hz before and during the application of NE, respectively ($n=10$), and 0.72 ± 0.27 and 0.63 ± 0.31 Hz in the presence and absence of 1 μM A61603, respectively ($n=8$).

These results suggest that (1) NE facilitates GABA release by a direct effect on GABAergic terminals, and (2) this mechanism of noradrenergic facilitation of GABA release is impaired by stress.

Facilitation of GABAergic Transmission by α_{1A} Adrenoceptors is Mediated by Phospholipase C (PLC)

Studies in other brain regions or cell types have shown that α_1 adrenoceptors are coupled to PLC via a G-protein, and can increase the intracellular calcium concentration $[Ca^{2+}]_i$ by mobilizing Ca^{2+} from intracellular stores, as well as by increasing the Ca^{2+} influx (Schwinn *et al*, 1991; Wu *et al*, 1992; Cohen and Almazan, 1993; Lepretre *et al*, 1994; Kulik *et al*, 1999). However, certain effects of α_{1A} activation involve signaling pathways that are independent of PLC activation and intracellular Ca^{2+} rise (Berts *et al*, 1999). To determine whether the α_{1A} adrenoceptor-mediated facilitation of GABA release, in the BLA, involves the activation of PLC, we examined whether the effects of NE on the GABAergic transmission are blocked by a PLC inhibitor. In control rats, NE (10 μM) or A61603 (1 μM) enhanced the frequency and amplitude of sIPSCs in the presence of U73343 (20 μM), the inactive isomer of the PLC inhibitor U73122, but had no effects in the presence of 20 μM U73122 (Figure 9). Thus, in the presence of U73343, NE increased the frequency of sIPSCs to 1022.8 ± 105.3% of the control levels ($p<0.01$, $n=8$; Figure 9a) and increased the amplitude of sIPSCs to 161 ± 11.7% ($p<0.01$, $n=6$; Figure 9a); A61603 (1 μM) increased the frequency of sIPSCs to 978.1 ± 102.1% ($p<0.01$, $n=8$; Figure 9b), and increased the amplitude of sIPSCs to 154 ± 12.3% of the control levels ($p<0.01$, $n=8$; Figure 9b). In contrast, in the presence of U73122 (20 μM), NE (10 μM) and A61603 (1 μM) failed to induce any significant changes in the frequency



and amplitude of sIPSCs (Figure 9c–e). Similarly, the effects of NE (10 μM) on the amplitude of eIPSCs, as well as on the frequency of mIPSCs, were blocked by 20 μM U73122 (not shown).

Stress Blocks α_{1A} Adrenoceptor-Mediated Suppression of BLA Field Potentials

Since the activation of α_{1A} adrenoceptors facilitates GABAergic transmission, the function of these receptors at the network level could be to dampen neuronal excitability and responsiveness. However, while spontaneous GABAergic activity is dramatically enhanced by activation of α_{1A} adrenoceptors (Figure 5), evoked GABAergic transmission is suppressed due to presynaptic inhibition of GABA release via $GABA_B$ autoreceptors (Figure 6). Therefore, under physiological conditions when $GABA_B$ receptors are not blocked, α_{1A} adrenoceptor activation could enhance the amygdala's responsiveness (due to the reduction in evoked GABA release), unless the enhancement of spontaneously released extracellular GABA plays a more decisive role in neuronal excitability. To determine the net effect of α_{1A} adrenoceptor activation on neuronal responsiveness and excitability in the BLA, and whether this effect is altered by stress, we investigated the effects of NE or A61603 on population, field responses, in the absence of $GABA_B$ receptor blockade, in control and stressed rats.

Field potentials in the BLA were evoked by stimulation of the external capsule. These responses consist of one major, negative component that corresponds in time course to the EPSP recorded intracellularly from BLA pyramidal cells (Aroniadou-Anderjaska *et al*, 2001; Chen *et al*, 2003), and is mediated by AMPA/kainate receptors (Aroniadou-Anderjaska *et al*, 2001). In control rats, 10 μM NE, in the presence of propranolol (10 μM) and yohimbine (20 μM), produced a significant reduction in the peak amplitude of evoked field potentials (83.8 ± 5.3% of control levels, $n = 14$, $p < 0.05$; Figure 10a). Similarly, bath application of 1 μM A61603 caused a significant reduction in the peak amplitude of the field potentials to 83.1 ± 5.2% of the control levels ($p < 0.05$, $n = 12$; Figure 10b). In contrast, in stressed rats, neither NE (10 μM) nor A61603 (1 μM) had a significant effect on the amplitude of the field potentials (Figure 10, bottom panels).

These results suggest that the function of α_{1A} adrenoceptors in the BLA is to reduce neuronal excitability/responsiveness, and this function is impaired by stress.

Figure 8 Activation of α_{1A} adrenoceptors increases the frequency of mIPSCs in control rats, but not in stressed rats. mIPSCs were recorded in the presence of TTX (1 μM), D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). (a) Top traces: mIPSCs recorded from a BLA pyramidal neuron of a control rat. NE (10 μM) increased the frequency of mIPSCs. Bottom graph: the left panel shows the cumulative probability plots of interevent intervals of mIPSCs under control conditions and during the application of NE (same cell as in top traces). The bar graph shows the effect of NE on the amplitude, kinetics, and frequency of mIPSCs. Pooled data from 10 neurons, $^{**}p < 0.01$. (b) Similar data to those shown in (a), but from stressed rats. NE had no significant effect on mIPSCs. The bar graph shows pooled data from 10 neurons. (c) In control rats, the α_{1A} antagonist A61603 had similar effects to those induced by NE. The bar graph shows pooled data from nine BLA neurons. (d) A61603 had no significant effects on mIPSCs recorded from BLA pyramidal cells of stressed rats. The bar graph shows pooled data from eight cells.

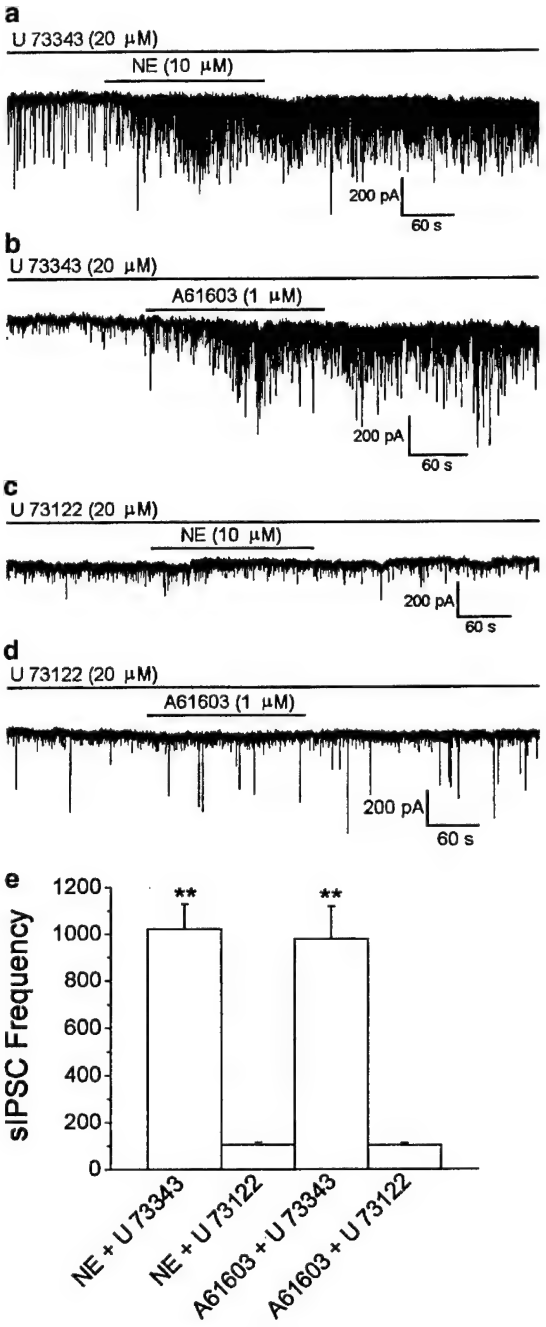


Figure 9 α_{1A} adrenoceptors in the BLA are coupled to PLC. (a–d) sIPSCs recorded from BLA pyramidal neurons. NE (a) or A61603 (b) increased the frequency and amplitude of sIPSCs in the presence of the inactive isomer of a PLC inhibitor (U73343), but had no effect in the presence of the PLC inhibitor U73122 (c and d). The slice medium contains D-AP5 (50 μM), CNQX (10 μM), propranolol (10 μM), and yohimbine (20 μM). (e) Bar graphs showing the effects of NE (10 μM) or A61603 (1 μM) on the frequency of sIPSCs in the presence of U73343 or U73122. Pooled data from eight neurons.

DISCUSSION

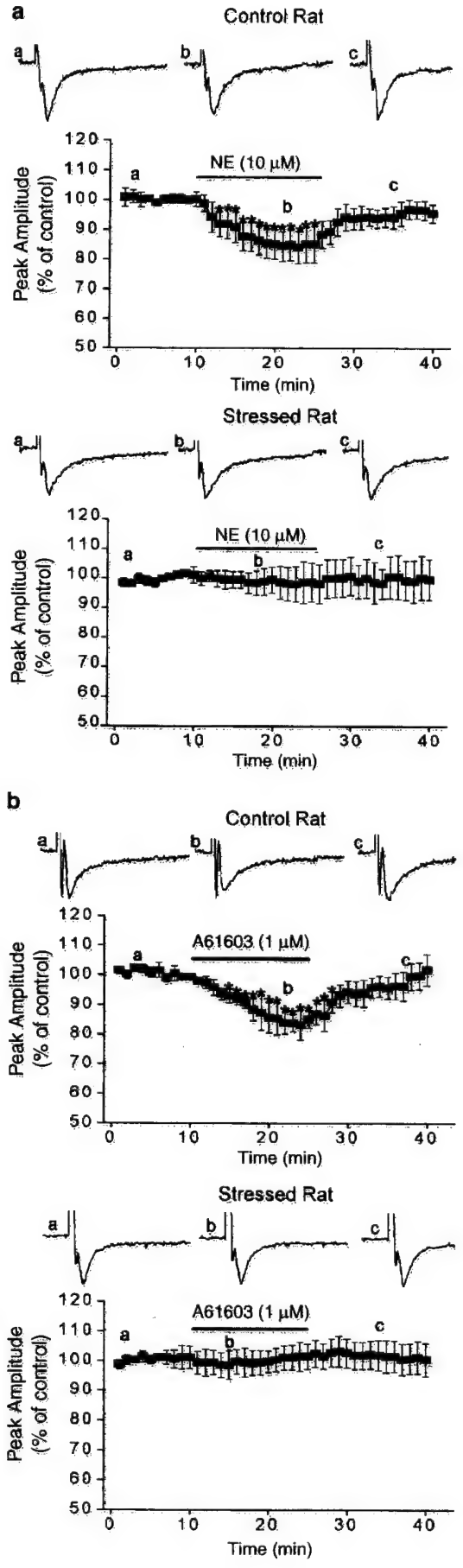
The present study describes two main findings. First, activation of the α_{1A} subtype of adrenergic receptors facilitates both tonic and phasic $GABA_A$ receptor-mediated inhibition of BLA pyramidal neurons. Second, stress produces a severe impairment of the α_{1A} adrenoceptor-mediated facilitation of GABAergic synaptic transmission in the BLA. These findings provide one possible explanation for (1) the antiepileptic effects of NE in temporal lobe epilepsy, (2) the amygdala's hyperexcitability in stress-related affective disorders, and (3) the stress-induced increase in the frequency of seizures in epileptic patients.

NE Facilitates GABAergic Transmission in the BLA via presynaptic α_{1A} Adrenoceptors

All three subtypes of α_1 adrenoceptors, α_{1A}, α_{1B}, and α_{1D}, are present in the amygdala, as determined by *in situ* hybridization (Day *et al*, 1997). The distribution of these receptors varies in different nuclei of the amygdala. The BLA expresses the α_{1A} adrenoceptor subtype almost exclusively (Day *et al*, 1997; Domyancic and Morilak, 1997). The role of these receptors in the amygdala's physiology and function has been unknown. In the present study, we show that NE, acting via the α_{1A} subtype of adrenergic receptors, facilitates GABA release in the BLA. Spontaneous, evoked, and quantal release of GABA were enhanced by NE or the specific α_{1A} adrenoceptor agonist A61603.

Endogenous NE released from noradrenergic terminals reaches its targets both by diffusion and via conventional synapses (Papadopoulos and Parnavelas, 1990; Seguela *et al*, 1990; Asan, 1993; Arce *et al*, 1994; Li *et al*, 2002). In the BLA, noradrenergic axons form asymmetric synapses with the dendrites of GABAergic neurons (Li *et al*, 2002). Although α_{1A} adrenoceptors may be located in such dendritic synapses and could be involved in the enhancement of spontaneous and evoked GABA release by NE, the increase in the frequency of mIPSCs by α_{1A} adrenoceptor activation indicates the presence of these receptors on GABAergic terminals. The enhancement of spontaneous GABA release by NE has also been observed in other brain regions (Madison and Nicoll, 1988; Doze *et al*, 1991; Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Bennett *et al*, 1997, 1998; Kawaguchi and Shindou, 1998), and it is mediated via α_1 adrenoceptors (Gellman and Aghajanian, 1993; Alreja and Liu, 1996; Bergles *et al*, 1996; Kawaguchi and Shindou, 1998); the specific α_1 receptor subtype involved has not been determined. At least in the CA1 hippocampal area, it appears that α_1 adrenoceptors are located only on somatodendritic regions of GABAergic cells, since mIPSCs are unaffected by adrenergic agonists (Bergles *et al*, 1996). Thus, the amygdala and the hippocampus may differ in the subcellular distribution of α_1 adrenoceptors mediating the facilitation of GABA release.

Evoked GABA release in the hippocampus is suppressed by NE, and this effect is also mediated via α adrenoceptors (Madison and Nicoll, 1988). However, a similar effect of NE in the sensorimotor cortex has been found to be due to the activation of presynaptic $GABA_B$ autoreceptors; when $GABA_B$ receptors were blocked, NE enhanced evoked GABAergic transmission (Bennett *et al*, 1997). Similarly, in the present study, the facilitatory effect of NE on evoked GABAergic transmission was revealed only when $GABA_B$ receptors were blocked, suggesting that the accumulation of extracellular GABA due to the NE-induced enhancement of spontaneous GABA release inhibited evoked GABA release.



Since NE enhances spontaneous GABA release, but suppresses evoked GABA release when $GABA_B$ receptors are functional, this raises the question of what would be the net effect of α_{1A} adrenoceptor activation on the overall excitability and responsiveness of the amygdala. The BLA field potentials were reduced by NE or A61603 in the absence of $GABA_B$ receptor antagonists. It is unlikely that this effect is due to a reduction in glutamate release, because glutamatergic transmission in the BLA is suppressed via α_2, but not α_1 adrenoceptor activation (Ferry *et al*, 1997). Thus, the reduction of the BLA field potentials by NE or A61603 suggests that the dramatic enhancement of spontaneously released GABA induced by α_{1A} adrenoceptor activation (Figure 5) over-rides the reduction in evoked GABAergic transmission (Figure 7) producing a suppression of the amygdala's excitability.

The intracellular signaling mechanisms that mediate the physiological effects of α_{1A} adrenoceptor activation in the BLA involve the activation of PLC, since a PLC inhibitor prevented the enhancement of sIPSCs, eIPSCs, and mIPSCs by NE and A61603. The activation of PLC may lead to mobilization of Ca^{2+} from intracellular stores, and/or Ca^{2+} influx, following phosphoinositide hydrolysis and formation of IP3, as it has been observed in different tissues and cell types following α_1 adrenoceptor activation (Schoepp and Rutledge, 1985; Schwinn *et al*, 1991; Perez *et al*, 1993; Kulik *et al*, 1999; Zhong and Minneman, 1999; Khorchid *et al*, 2002), or α_{1A} adrenoreceptor activation (Cohen and Almazan, 1993; Lepretre *et al*, 1994). In the present study, since NE or A61603 enhanced the frequency of mIPSCs, the influx of Ca^{2+} through voltage-gated calcium channels is not necessary for the α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA.

The amygdala is a key player in the pathogenesis and symptomatology of temporal lobe epilepsy (Gloor, 1992; Weiss *et al*, 2000; Avoli *et al*, 2002). NE has long been known to display anticonvulsant properties, but little is known about the underlying mechanisms (Chen *et al*, 1954; Stanton, 1992; Stanton *et al*, 1992; Szot *et al*, 1999; Stoop *et al*, 2000; Weinshenker *et al*, 2001) The α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA may be one of the mechanisms involved in the antiepileptic effects of NE in temporal lobe seizure disorders.

Stress Impairs the Function of α_{1A} Adrenoceptors in the BLA

Previous studies have suggested that excessive or repeated stress can produce long-lasting alterations in the amygdala's structure and function. Thus, chronic immobilization, in rats, induces hypertrophy of the dendritic arborizations of

Figure 10 Activation of α_{1A} adrenoceptors reduces BLA field potentials in control rats, but not in stressed rats. (a) Changes in the peak amplitude of BLA field potentials evoked by stimulation of the external capsule, in response to bath application of 10 M NE, in control (top panel, $n=9$) and stressed (bottom panel, $n=10$) rats. The medium contains propranolol (10 M) and yohimbine (20 M). (b) Similar data to those in (a), except that A61603 is applied in place of the NE. Pooled data from 10 slices (control rats, top panel) and eight slices (stressed rats, bottom panel). The slice medium same as in (a). Asterisks over error bars denote statistically significant reduction ($p<0.05$).

pyramidal and stellate neurons in the BLA (Vyas *et al*, 2002; Pawlak *et al*, 2003). Fear conditioning or other types of stressors such as exposure to a predator produce long-lasting changes in the efficacy of synaptic transmission in the amygdala (LeDoux, 1992; Davis *et al*, 1994; Rogan *et al*, 1997, McKernan and Shinnick-Gallagher, 1997; Adamec *et al*, 2001). In human patients with stress-related affective disorders, the amygdala exhibits hypertrophy (Strakowski *et al*, 1999; Altshuler *et al*, 2000), increased levels of basal activity (Drevets, 1999), or exaggerated responses to fearful stimuli (Rauch *et al*, 2000). In the present study, repeated restrain/tail-shock stress produced a severe impairment in the α_{1A} adrenoreceptor-mediated facilitation of GABA release in the BLA, indicating that stress impairs the function of α_{1A} adrenoceptors. This impairment could result from receptor desensitization, internalization, or down-regulation, or by an effect on the intracellular signaling pathways activated by PLC. In other brain regions, repeated stress reduces mRNA levels of α_1 adrenoceptors (Miyahara *et al*, 1999). Adrenergic receptors desensitize or undergo downregulation following prolonged exposure to the agonist (Yang *et al*, 1999; Chalothorn *et al*, 2002). Thus, during stress exposure, excessive release of NE in the amygdala (Galvez *et al*, 1996; Quirarte *et al*, 1998; Tanaka *et al*, 2000) may be responsible for the impairment of the α_{1A} adrenoreceptor function. In addition, previous studies have shown that restrain/tail-shock stress elevates plasma corticosterone levels (Servatius *et al*, 1995). Glucocorticoid receptors colocalize with α_1 adrenoceptors (Fuxe *et al*, 1985; Williams *et al*, 1997), and it has been demonstrated that corticosterone downregulates α adrenoceptors (Stone *et al*, 1986, 1987; Joels and de Kloet, 1989). Therefore, another possibility is that the corticosterone released during exposure to stress downregulates α_{1A} adrenoceptors. An important question is whether the impairment in the α_{1A} adrenoceptor function is a transient or a long-term effect. The investigations described here focus on changes measured within a relatively short period of time after stressor cessation. However, preliminary experiments have revealed differences in the α_{1A} adrenoceptor function between stressed and control rats on the fifth day after the termination of stress exposure, suggesting that the stress-induced dysfunction in the noradrenergic modulation of GABA release is not likely to be a short-term effect.

Functional implications. What are the possible functional implications of a stress-induced loss of the α_{1A} adrenoceptor-mediated noradrenergic facilitation of GABA release in the BLA? In the normal amygdala, basal levels of NE, acting via α_{1A} adrenoceptors, may contribute to tonic inhibition of BLA pyramidal neurons, by facilitating both action potential-dependent and -independent GABA release. The loss or impairment of this facilitation would result in hyperexcitability at rest, and a lower threshold of activation. When the normal amygdala is activated in response to an emotionally significant event triggering the release of NE, activation of α_{1A} adrenoceptors will facilitate the role of inhibitory transmission in active neuronal circuits; this role is not only to prevent overexcitation, but also to shape and sharpen the flow of excitatory activity. Therefore, loss of the α_{1A} adrenoceptor-mediated facilitation of synaptic inhibition may result in inappropriate overactivation of the amygdala and impairment in the processing and interpretation of an emotional stimulus. A dysfunction of this nature may also affect the formation of emotional memories. In the normal amygdala, noradrenergic facilitation of GABAergic transmission may either suppress memory formation (due to the suppression of excitation), or facilitate optimal registration of the memory trace (by regulating the level and flow of excitatory activity). In a hyper-responsive amygdala, when noradrenergic facilitation of GABA release is impaired, events of little emotional significance may be registered as significant, and memories of emotionally significant events may be 'overconsolidated'. It should be noted, however, that the net effect of stress on the function of the noradrenergic system in the BLA remains to be determined, as stress may also induce changes in the interaction of NE with other adrenoceptor subtypes (β and α_2) or neurotransmitter systems.

It has been hypothesized that the hyperactivity and hyper-responsiveness of the amygdala associated with certain affective disorders, such as PTSD, is due to the loss of proper cortical modulation of the amygdala, and/or due to an intrinsic lower threshold of amygdala response to emotionally significant stimuli (Villarreal and King, 2001). The present findings suggest that a reduction in GABAergic transmission due to the loss of the α_{1A} adrenoceptor-mediated facilitation of GABA release may be one of the mechanisms responsible for the apparently reduced threshold of amygdala's activation in these affective disorders. The present findings also suggest that a stress-induced impairment in the function of α_{1A} adrenoceptors, which could result in reduced tonic inhibition in the BLA, may be one of the mechanisms underlying the stress-induced increased frequency of seizures in patients with temporal lobe epilepsy (Temkin and Davis, 1984; Frucht *et al*, 2000). Moreover, our results suggest that the reduced central α_1 adrenoceptor responsiveness (Asnis *et al*, 1985, 1992), and binding (Crow *et al*, 1984; Gross-Isseroff *et al*, 1990) in depressed patients may be stress-related, and that one of the physiological consequences of this reduction is an impaired modulation of the GABAergic transmission.

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APPENDIX 4

THE PHYSIOLOGICAL ROLE OF KAINATE RECEPTORS IN THE AMYGDALA

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ABSTRACT

The kainate subtype of glutamate receptors has received considerable attention in recent years, and a wealth of knowledge has been obtained in regard to the function of these receptors. Kainate receptors have been shown to mediate synaptic transmission, in some brain regions, modulate presynaptic release of glutamate and GABA, and mediate synaptic plasticity, or the development of seizure activity. This review focuses on the function of kainate receptors in the amygdala, a brain region that plays a central role in emotional behavior and certain psychiatric illnesses. Evidence is reviewed indicating that postsynaptic kainate receptors containing the GLU_{k5} subunit are present on interneurons and pyramidal cells in the basolateral amygdala, and mediate a component of the synaptic responses of these neurons to glutamatergic input. In addition, GLU_{k5}-containing kainate receptors are present on presynaptic terminals of GABAergic neurons, where they modulate the release of GABA in an agonist concentration-dependent, bi-directional manner. GLU_{k5}-containing kainate receptors also mediate a long-lasting synaptic facilitation, induced by low-frequency stimulation in the external capsule to the basolateral nucleus pathway, and they appear to be partly responsible for the susceptibility of the amygdala to epileptogenesis. Taken together, these findings have suggested a prominent role of GLU_{k5}-containing kainate receptors in the regulation of neuronal excitability in the amygdala.

INTRODUCTION

Fast excitatory neurotransmission in the vertebrate central nervous system (CNS) is mediated primarily by glutamate. Based on pharmacological studies using selective agonists, ionotropic glutamate receptors have been divided into three major classes of receptor subtypes: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors. Subsequent molecular cloning of subunits that form glutamate receptors has confirmed the validity of this pharmacological subdivision and has greatly enhanced our understanding of their functional properties.

Kainate receptors consist of five different subunits, namely GLU_{k5}, GLU_{k6}, GLU_{k7}, GLU_{k1}, and GLU_{k2} (review recently by 1-2). GLU_{k5-7} subunits form homomeric and heteromeric functional channels when expressed in heterologous systems (3-8). GLU_{k1}, and GLU_{k2} subunits do not form functional homomers in the same systems, but generate functional receptors with distinct physiological properties when combined with GLU_{k5}, GLU_{k6}, or GLU_{k7} subunits (6,9-10). Consequently, a large number of distinct kainate receptor subtypes could be assembled based on the combinatorial possibilities of these five different subunits. In addition, kainate receptor subunits are subjected to both alternative splicing and RNA editing, which significantly increase the number of subunit isoforms. Alternative splicing has been reported for GLU_{k5}, GLU_{k6} and GLU_{k7} subunits (3-4,6,11-12), but the role of the different splice variants is unknown. Post-transcriptional mRNA editing has been described for the GLU_{k5} and GLU_{k6} subunits at the Q/R site of the M2 domain (13,14), which decreases the permeability to calcium (5,15) and transforms the rectification properties of these receptors from inwardly rectifying to linear

or slightly outwardly rectifying (14,16-20). GLU_{k6} can also undergo further editing at two additional sites in the M1 domain (21), however the role of the M1 editing sites remains unknown.

Although little is known about the precise subunit composition of native kainate receptors, their potential compositional diversity becomes evident by the numerous, distinct physiological roles that these receptors seem to play in the CNS. Kainate receptors have been shown to mediate fast excitatory synaptic transmission (22-26), modulate transmitter release at both excitatory and inhibitory synapses (reviewed by 27-29), and to be involved in short- and long-term synaptic plasticity mechanisms (review recently by 1-2).

While kainate receptor subunit genes are widely expressed throughout the brain (30), it appears that, in different brain regions, kainate receptors may have different functions. Their function may ultimately depend on their cellular and subcellular localization, subunit composition and stoichiometry, as well as density. This review addresses the functional roles of kainate receptors in the amygdala, a brain region that plays a central role in all aspects of emotional behavior, such as emotional learning and memory functions (31-34), responses to psychological stress (35-37), as well as pathophysiological conditions such as those associated with affective disorders (38-42) or temporal lobe epilepsy (43).

KAINATE RECEPTORS ARE HIGHLY EXPRESSED IN THE AMYGDALA

The amygdaloid complex is a group of more than ten nuclei that are located in the midtemporal lobe, and have extensive internuclear and intranuclear connections (44). The

amygdala receives information from all sensory modalities, via glutamatergic excitatory inputs from the cerebral cortex, the thalamus, and other subcortical brain regions (44-45). Glutamate is also the major excitatory neurotransmitter in intra-amygdala circuits (46-51). It has been previously shown that glutamatergic synapses in the amygdala express NMDA, AMPA and kainate receptors (for a review see 45).

In situ hybridization studies have revealed that certain kainate receptor subunits are highly expressed in the amygdala (Fig. 1). Thus, mRNA levels of GLU_{k5}, GLU_{k6} and KLU_{k2} subunits are high in most regions of the amygdala (52). The GLU_{k5} subunit in particular is higher in the amygdala than in the hippocampus, and it is mainly concentrated in the basolateral and medial nuclei (52-53).

The heavy expression of kainate receptors in the amygdala may imply a prominent physiological role of these receptors in this brain region. Indeed, there is already evidence that kainate receptors in the amygdala 1) mediate a component of excitatory synaptic transmission, 2) modulate the release of GABA in interneurons to pyramidal cell synapses, 3) play an important role in certain forms of amygdala's synaptic plasticity, and 4) are significantly involved in certain pathophysiological conditions of the amygdala, such as temporal lobe epilepsy.

POSTSYNAPTIC GLU_{K5} KAINATE RECEPTORS MEDIATE EXCITATORY SYNAPTIC TRANSMISSION IN THE AMYGDALA

Early evidence for kainate receptor-mediated excitatory synaptic responses came from observations in the hippocampal mossy fiber synapses (22,54). These studies became possible when pharmacological tools capable of selectively blocking AMPA or

kainate receptors became available (22,54-57). Subsequently, kainate receptor-mediated synaptic responses have been reported in cerebellar Golgi cells (58), at thalamocortical synapses (26), in sensory fiber-dorsal horn neurons in the spinal cord (59), and in the basolateral nucleus of the amygdala (BLA, 25,53).

Most studies on the function of kainate receptors in the amygdala have been focused on the BLA. The BLA, along with the lateral nucleus, are the entry sites for afferent inputs to the amygdala (45). In the BLA, the bulk (about 70%, 25) of the glutamatergic excitatory postsynaptic responses is mediated by AMPA receptors. However, using selective pharmacological antagonists, Li and Rogawski (1998) first demonstrated that a component (about 30%) of the excitatory postsynaptic potential (EPSP) evoked by the stimulation of the external capsule (EC) in BLA neurons is mediated by kainate receptors (25). Thus, it was found that a component of the EPSP was resistant to NMDA receptor antagonists and to the AMPA receptor-selective, allosteric antagonists GYKI 52466 and GYKI 53655. This component was blocked by the GLU_{k5}-selective kainate receptor antagonist LY293558, suggesting that it was mediated by GLU_{k5} containing kainate receptors (Fig. 2). Like in the hippocampus, the GLUk5-mediated EPSP showed a remarkable dependence on the stimulation frequency. Increasing the stimulation frequency of the EC produced a large increase in the amplitude of the kainate receptor-mediated synaptic responses (Fig. 2).

Subsequently, Braga et al. (53) found that a specific GLU_{k5} kainate receptor agonist enhances the frequency and amplitude of TTX-sensitive, spontaneous GABAergic currents (IPSCs) recorded from BLA pyramidal cells (Fig. 3B). This observation suggested that GLU_{k5} kainate receptor activation depolarizes inhibitory

interneurons. To determine whether postsynaptic GLU_{k5} kainate receptors are indeed present on BLA interneurons, excitatory postsynaptic currents (EPSCs) evoked by electric stimulation of the external capsule (3 shocks delivered at 100 Hz, every 10 s) were recorded from identified BLA interneurons, in the presence of GYKI 53655 (50 μM), D-APV (50 μM), bicuculline (10 μM), and SCH50911 (20 μM), to block AMPA, NMDA, $GABA_A$, and $GABA_B$ receptors, respectively, These evoked EPSCs were completely blocked by bath application of LY293558, suggesting that they were mediated by GLU_{k5} kainate receptors (Fig. 3A). GLU_{k5} kainate receptor-mediated EPSCs have also been recorded from BLA pyramidal neurons (60). Thus, it appears that, in the BLA, GLU_{k5} kainate receptors are present on somatodendritic regions of both pyramidal cells and interneurons, and mediate a component of the evoked EPSCs.

BIDIRECTIONAL MODULATION OF GABA RELEASE BY PRESYNAPTIC GLU_{k5} KAINATE RECEPTORS IN THE BLA

In addition to mediating excitatory synaptic transmission in some brain regions, kainate receptors have been shown to modulate the release of glutamate and GABA (reviewed by 27-28). In both excitatory and inhibitory synapses, kainate receptors were initially found to depress neurotransmitter release (28). More recent studies demonstrated that kainate receptor activation can also facilitate transmitter release (61-64)).

In the BLA, Braga et al. (53) showed that GLU_{k5} kainate receptors are present on presynaptic GABAergic terminals contacting pyramidal cells, and that activation of these receptors bidirectionally modulates the release of GABA, in an agonist-concentration dependent manner. Thus, low concentrations of the specific GLU_{k5} kainate receptor

agonist, ATPA, or glutamate (0.3 and 5 μM, respectively) potentiated evoked GABA release, while high concentratrions of the agonists (10 μM ATPA or 200 μM glutamate) depressed it. These effects were unrelated to activation of $GABA_B$ or group I metabotropic glutamate receptors, as they persisted in the presence of SCH 50911 and CPCCOEt. Low concentrations of ATPA or glutamate also increased the frequency of miniature inhibitory postsynaptic currents, while high concentrations of these agonists reduced it (Fig. 4). The effects of the GLU_{k5} kainate receptor agonists on the TTX-insensitive release of GABA did not require activation of voltage-dependent Ca^{2+} channels, or $GABA_B$ receptors, or group I metabotropic glutamate receptors. The same study provided evidence that endogenous glutamate gains access to presynaptic GLU_{k5} kainate receptors that are present on inhibitory terminals, and tonically facilitates evoked GABA release.

These findings led to the hypothesis that the terminals of GABAergic neurons in the BLA contain two subtypes of GLU_{k5}-bearing kainate receptors, which have different affinities to their agonists, and activate different mechanisms of action. Based on their affinity for [^{3}H]kainate, kainate receptor subunits can be divided into low-affinity (GLU_{k5}, GLU_{k6} and GLU_{k7}) and high-affinity (GLU_{k1} and GLU_{k2}) subunits (65). The BLA expresses high levels of the GLU_{k6} and GLU_{k2} subunit mRNAs, in addition to GLU_{k5} (52). There is evidence that the GLU_{k5} subunit can form functional kainate receptors with GLU_{k6} or GLU_{k2} subunits, and both GLU_{k5}/GLU_{k6} and GLU_{k5}/GLU_{k2} kainate receptors are sensitive to ATPA (8). Therefore, a GLU_{k5}/GLU_{k2} and a GLU_{k5}/GLU_{k6} subunit combination could, respectively, mediate the facilitation and inhibition of GABAergic transmission in the BLA. Consistent with the view that a

GLU_{k5}/GLU_{k6} subunit combination may mediate the suppression of GABAergic transmission in the BLA, Mulle et al (66) found that kainate-induced suppression of evoked IPSCs in the hippocampus is mediated by heteromeric kainate receptors composed of both GLU_{k5} and GLU_{k6} subunits.

The intracellular mechanisms to which these presynaptic GLU_{k5} receptors are coupled remain to be elucidated. In the hippocampus, evidence exists for the participation of both metabotropic and ionotropic cascades following the activation of kainate receptors (62,66-69). The agonist concentration-dependent, bi-directional modulation of GABA release via presynaptic GLU_{k5} kainate receptors in the BLA, suggests a significant role of glutamate diffusion in the regulation of neuronal excitability in this brain region. Low concentrations of extracellular glutamate escaping from excitatory synapses during tonic or low-level activity of excitatory pathways in the BLA can be expected to facilitate GABAergic transmission. Considering the central role of the amygdala, and the BLA in particular, in fear-conditioning and consolidation of emotional memories (70) such facilitation of GABAergic transmission may serve to prevent or dampen excitation of the amygdala during external or internal stimuli that have only modest emotional significance. In contrast, in response to intense emotional stimuli that produce strong excitation of the amygdala, the amount of glutamate released may reach sufficiently high extrasynaptic concentrations to activate the low-affinity GLU_{k5} kainate receptors on GABAergic terminals, inhibiting evoked GABAergic transmission. Such an effect could further enhance overactivity in the amygdala during intense emotional stimuli, and perhaps facilitate the "registration" of the memory trace representing the emotional event. In that respect, this GLU_{k5}-mediated disinhibitory effect of glutamate

may play an important role in synaptic plasticity and memory formation in the amygdala, as well as in the development of certain stress-related affective disorders such as Post-Traumatic Stress Syndrome.

KAINATE RECEPTORS MEDIATE A FORM OF SYNAPTIC PLASTICITY IN THE AMYGDALA

Synaptic plasticity phenomena such as long-term potentiation (LTP) and long-term depression (LTD) are believed to be cellular mechanisms that underlie learning and memory processes (71-76). In all brain regions examined so far, the intracellular events that induce LTP or LTD are triggered by a rise in intracellular calcium, postsynaptically, and, in some synapses, presynaptically as well (72,77-81). In most forms of LTP and LTD, the mechanism by which intracellular free calcium increases is the influx of calcium via postsynaptic NMDA receptors (77,81-87). However, synaptic plasticity that does not require NMDA receptor activation has also been reported in many brain regions (79,87-92).

Various forms of synaptic plasticity have also been described in the amygdala (93-100). A novel form of synaptic plasticity whereby low-frequency stimulation (1 Hz for 15 min) of the external capsule (EC) induces a long-lasting synaptic facilitation of EPSPs recorded from BLA neurons (51), has been shown to be mediated by GLU_{k5} kainate receptors (52). Thus, induction of this form of synaptic potentiation (LFIF, for low-frequency induced facilitation) was blocked by antagonists that are selective for GLU_{K5} kainate receptors (LY377770 and LY382884), but not by antagonists of NMDA (100 μM APV), AMPA (50 μM GYKI53655) or group I metabotropic (20 μM

CPCCOEt) glutamate receptors. Furthermore, a similar form of lasting potentiation was induced by brief (10min) exposure of the amygdala to the GLU_{K5}-selective agonist ATPA (20 μM). An increase in intracellular calcium was necessary for the induction of the GLU_{k5} kainate receptor-mediated LFIF. Potentiation was expressed in both the NMDA and the AMPA/kainate receptor-mediated components of the EPSPs. Interestingly, potentiation was not restricted to the fibers stimulated during the induction period (homosynaptic potentiation), but instead was generalized to other converging pathways (heterosynaptic potentiation, 52).

The mechanisms by which GLU_{k5} kainate receptors mediate the induction of LFIF, and the mechanisms of heterosynaptic spread of this form of synaptic facilitation remain to be elucidated. GLU_{K5} kainate receptors can be permeable to calcium, particularly when they contain unedited kainate receptor subunits (65). About 30% of the GLUk5 subunits present in the BLA are in the unedited form (52), and, therefore they may participate in forming calcium permeable kainate receptors that contribute to synaptic plasticity. Whether the GLU_{k5}-containing receptors that mediate LFIF are present postsynaptically or presynaptically in not known. As mentioned earlier above, there is electrophysiological evidence that GLU_{k5} receptors are present on somatodendritic regions of both BLA pyramidal cells and interneurons. Whether they are also present on glutamatergic presynaptic terminals of afferent pathways remains to be determined. The enhancement of both the NMDA and AMPA/kainate components of the EPSP may suggest involvement of presynaptic mechanisms.

Kainate receptors desensitize rapidly (101). This may be one reason that, in general, they do not contribute significantly to the induction of LTP by high-frequency

stimulation. However, during low-frequency stimulation, these receptors may have sufficient time to recover from the desensitized state, and thus contribute to postsynaptic depolarization and calcium influx.

In the hippocampus, low-frequency stimulation induces LTD (102). One reason for this difference between the hippocampus and the EC to BLA pathway may be that the BLA has a substantially higher concentration of GLU_{k5} kainate receptors, and, therefore more calcium may enter postsynaptically (and/or presynaptically) during low-frequency stimulation, resulting in synaptic potentiation rather than depression.

KAINATE RECEPTORS AND TEMPORAL LOBE EPILEPSY

The amygdala plays a central role in temporal lobe epilepsy (43,103). It is a key structure in the generation of seizures, as well as in the spread of limbic seizure activity, through its connections with the entorhinal cortex and hippocampus (103). Little is known about the mechanisms that underlie amygdala's susceptibility to epileptogenesis. However, kainate receptors seem to play a significant role, since a single injection of kainic acid, a preferential kainate receptor agonist, into the amygdala produces cell damage and elicits chronic, spontaneous, recurrent epileptiform activity similar to that observed in human temporal lobe epilepsy. Recent evidence suggests that GLU_{k5} kainate receptors, in particular, may play an important role in amygdala's vulnerability (43). Thus, the selective GLU_{k5} kainate receptor agonist, ATPA, induces spontaneous epileptiform bursting in amygdala slices, and limbic status epilepticus when infused into the rat amygdala. The effects of ATPA are blocked by the GLU_{k5} kainate receptor antagonist LY293558. Additional evidence that GLU_{k5} kainate receptors are involved in

the generation of epileptic activity in the amygdala comes from the findings that the anticonvulsant topiramate inhibits GLU_{k5} kainate receptor-mediated synaptic currents in the BLA (60), which suggests that topiramate may protect against seizures, at least in part, through suppression of GLU_{k5} kainate receptor activity.

What could be the mechanisms by which GLU_{k5} kainate receptor agonists induce epileptic activity in the amygdala? As discussed above, current evidence suggests that GLU_{k5}-containing kainate receptors are present on somatodendritic sites of both pyramidal cells (60) and interneurons (53), as well as on presynaptic terminals of GABAergic interneurons (53). The action of GLU_{k5} agonists on somatodendritic regions of interneneurons will depolarize these cells, enhancing spontaneous GABA release, which would suppress amygdala's excitability. In contrast, the action of the GLU_{k5} agonists on somatodendritic regions of pyramidal cells will depolarize these cells, increasing glutamate release, which would to enhance amygdala's excitability. At the same time, the GLU_{k5} kainate receptor agonists are acting at GABAergic presynaptic terminals. When agonist concentrations are low, evoked GABA release will be enhanced, which will favor suppression of pyramidal cell excitability. In contrast, when agonist concentrations are sufficiently high, evoked GABA release will be suppressed, which will favor an enhancement of neuronal excitability. Field potential recordings have indicated that the net effect of low-level activation of GLU_{k5} kainate receptors (1 μM ATPA in the slice medium) is a suppression in the overall neuronal excitability in the BLA, whereas the net effect of strong activation of GLU_{k5} kainate receptors (10 μM ATPA) is an enhancement in overall neuronal excitability and generation of epileptiform

activity (Aroniadou-Anderjaska et al., unpublished observations). The effects of GLU_{k5} kainate receptors activation are summarized in Fig.5.

PERSPECTIVES

Understanding the physiology of the amygdala is central to understanding the neurobiological mechanisms underlying emotional behavior, as well as psychiatric illnesses such as affective disorders, including stress-related affective disorders whose incidence has substantially increased in recent years, or temporal lobe epilepsy. Knowledge of the mechanisms that regulate neuronal excitability in the amygdala is imperative in understanding the pathophysiology of these diseases, as well as in the discovery of new, effective treatment strategies. The prominent presence of kainate receptors in the amygdala suggests that these receptors may play a significant role in the amygdala's function. As discussed in this review, recent evidence indeed indicates that GLU_{k5}-containing kainate receptors play an important role in the regulation of amygdala's excitability. However, a number of questions remain to be answered before a complete view emerges in regard to the functions of GLU_{k5} kainate receptors in the amygdala. For example, it remains to be determined whether GLU_{k5} kainate receptors are present of excitatory synaptic terminals, regulating glutamate release. The presence of low- and high-affinity GLU_{k5}-containing kainate receptors on GABAergic terminals has to be confirmed by further studies, and the opposing intracellular signaling pathways that these receptors activate, producing suppression or enhancement of GABA release, remain to be investigated. The precise mechanisms by which GLU_{k5} kainate receptors mediate synaptic plasticity in the BLA also must be delineated. The role of other subtypes of

kainate receptors in amygdala's physiology, as well as the composition and stoichiometry of native kainate receptors await further study.

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FIGURE LEGENDS

Figure 1. Pseudocolor images of GLU_{k5}, GLU_{k6} and GLU_{k2} mRNA expression, as revealed by *in situ* hybridization in rat brain coronal sections at the level of the amygdala. Although GLU_{k6} and GLU_{k2} mRNA signal is strongest in the hippocampus, GLU_{k5} mRNA expression is highest in the amygdala.

Figure 2. GLU_{k5} receptors mediate a component of the EPSP evoked in BLA neurons by stimulation of the external capsule (EC). The AMPA receptor antagonist GYKI-53655 (50 μM) reduced the EPSP evoked by single-pulse stimulation of the EC (2). High frequency stimulation (6 pulses at 100 Hz) substantially increased the magnitude of the residual, GYKI 53655-resistant EPSP, which was subsequently blocked by the GLU_{k5} antagonist LY293558 (10 μM). The slice medium contains the NMDA receptor antagonist APV (100 μM) and the $GABA_A$ receptor antagonist bicuculline (10 μM). The recording electrode contains 50 mM QX-314.

Figure 3. Excitation of BLA interneurons via GLU_{k5} kainate receptors. (Aa) GLU_{k5} kainate receptors mediate a component of the synaptic responses of BLA interneurons. Excitatory postsynaptic currents (Vh -60 mV) recorded from a BLA interneuron in the presence of GYKI 53655 (50 μM), D-APV (50 μM), bicuculline (10 μM), and SCH50911 (20 μM). Electrical stimulation was applied to the external capsule (3 shocks delivered at 100 Hz, every 10 s). The EPSC was blocked by the GLU_{k5} antagonist LY293558. A photomicrograph of the interneuron recorded in (**a**) is shown (**b**). Scale bar = 50 μm. (**Ba**) Activation of GLU_{k5} kainate receptors increases spontaneous activity of

BLA interneurons. Effects of different concentrations of ATPA on spontaneous IPSCs (sIPSCs) recorded from the soma of three different BLA pyramidal neurons (Vh +10 mV). A photomicrograph of one of these neurons is shown in **(b)**. Scale bar = 100 μm.

Figure 4. Dose-dependent, bidirectional modulation of the frequency of miniature GABAergic currents by the GLU_{k5} agonist ATPA. Traces in (A) and (B) are samples of miniature IPSCs (mIPSCs) recorded from two different BLA pyramidal neurons before, during, and after application of 300 nM (panel A), or 10 μM (panel B) ATPA in the presence of Cd^{2+} (100 μM), TTX (1 μM), GYKI 53655 (50 μM), D-APV (50 μM), and SCH50911 (20 μM), at a holding potential of +10 mV. Top plots show the effects of 300 nM (panel A) and 10 μM (panel B) ATPA on the mean frequency of mIPSCs as a function of time (bin = 60 s). Bottom bar graphs show pooled data (mean ± SEM). At 300 nM (panel A), ATPA increased the frequency of mIPSCs ($n = 6$, $^*p<0.05$). At 10 μM (panel B), ATPA produced a marked reduction in the frequency of mIPSCs ($n = 3$, $^*p<0.05$). For each cell, mIPSC frequency was normalized to the value of mean mIPSC frequency before application of ATPA. Co-application of LY293558 (30 μM) prevented the effects of ATPA.

Figure 5. Schematic representation of the agonist concentration-dependent bidirectional modulation of neuronal excitability by GLU_{k5} receptors, in the BLA. Physiological studies have suggested the presence of GLU_{k5}-containing kainate receptors on somatodendritic sites of both pyramidal cells and interneurons, as well as on presynaptic terminals of GABAergic interneurons. GABAergic terminals appear to carry

two subtypes of GLU_{k5}-containing kainate receptors, which have different affinities for glutamate and activate opposing mechanisms of action. Low concentrations of GLU_{k5} kainate receptor agonists will depolarize both pyramidal cells and interneurons (via somatodendritic receptors), and will increase evoked GABA release (and mIPSCs) via activation of the high-affinity, presynaptic GLU_{k5} kainate receptors. The result is a substantial increase in GABA release which may suppress excitability of the BLA neuronal network. High concentrations of GLU_{k5} kainate receptor agonists will again depolarize both interneurons and pyramidal cells, and will suppress evoked GABA release (and mIPSCs) via activation of the low-affinity, presynaptic GLU_{k5} kainate receptors. The result is likely to be an enhancement in the excitability of the BLA neuronal network. These hypotheses in regard to the net effects of low or high agonist concentrations have been supported by field potential recordings (see text). p, pyramidal cell; i, interneuron; eIPSC, evoked inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; sIPSP, spontaneous inhibitory postsynaptic current.

Figure 1

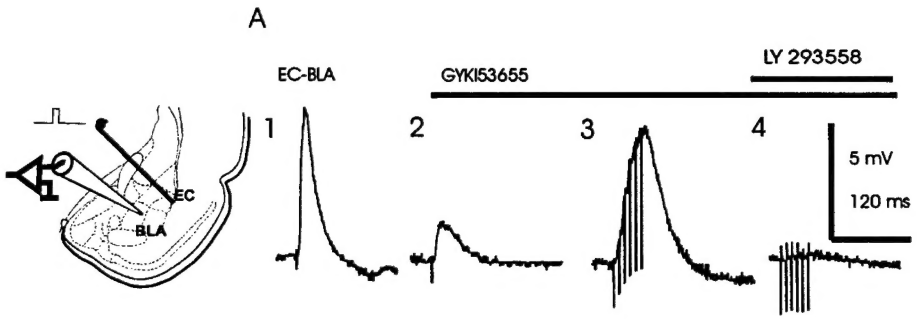

Figure 2

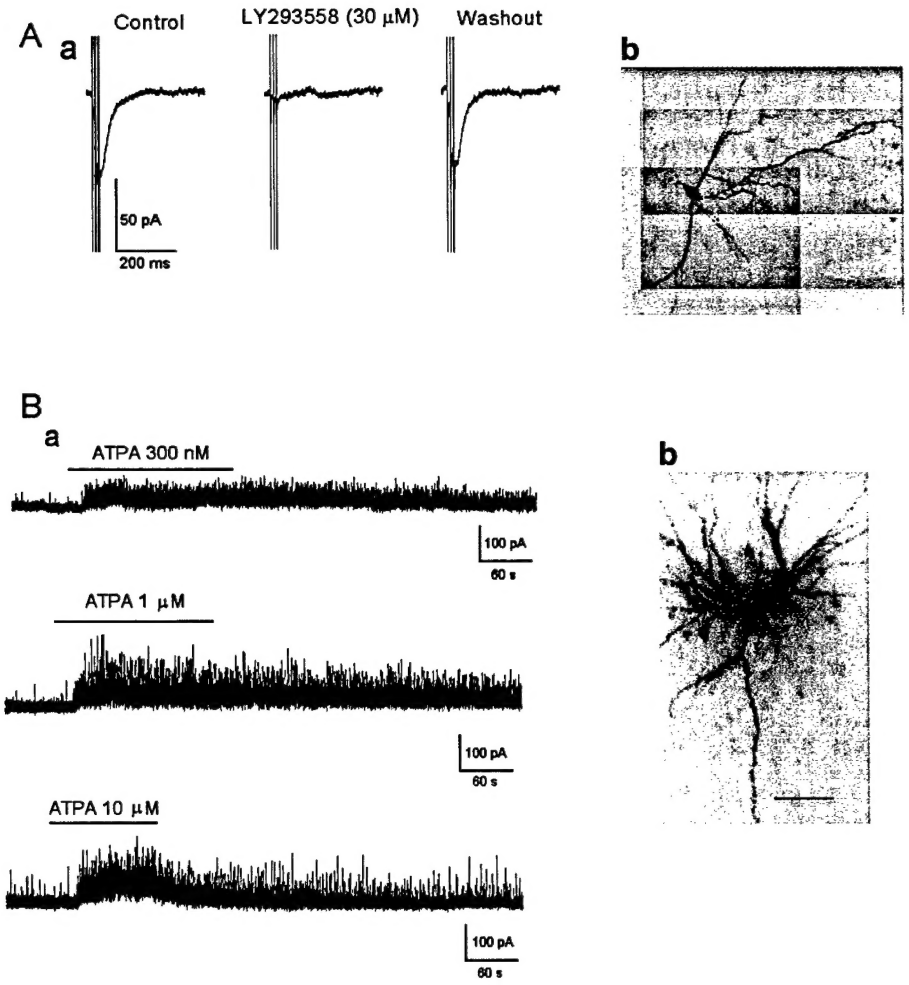
A
a
Control
LY293558 (30 µM)
Washout
50 pA
200 ms
b
B
a
ATPA 300 nM
100 pA
60 s
ATPA 1 µM
100 pA
60 s
ATPA 10 µM
100 pA
60 s
b

Figure 3

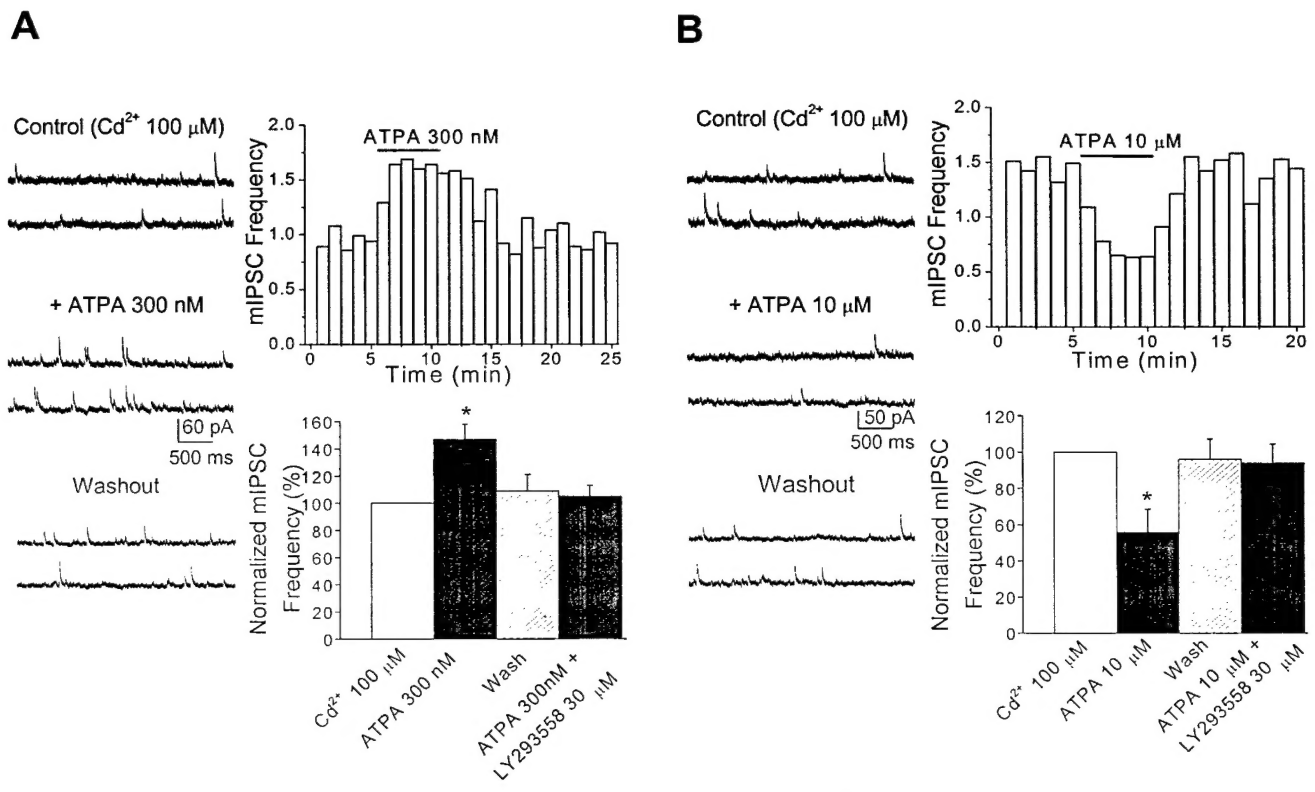
A
Control (Cd2+ 100 μM)
+ ATPA 300 nM
60 pA
500 ms
Washout
ATPA 300 nM
mIPSC Frequency
Time (min)
Normalized mIPSC Frequency (%)
Cd2+ 100 μM
ATPA 300 nM
Wash
ATPA 300nM + LY293558 30 μM
B
Control (Cd2+ 100 μM)
+ ATPA 10 μM
50 pA
500 ms
Washout
ATPA 10 μM
mIPSC Frequency
Time (min)
Normalized mIPSC Frequency (%)
Cd2+ 100 μM
ATPA 10 μM
Wash
ATPA 10 μM + LY293558 30 μM

Figure 4

Bidirectional modulation of GABAergic inhibition by GluR5 kainate receptors in the BLA

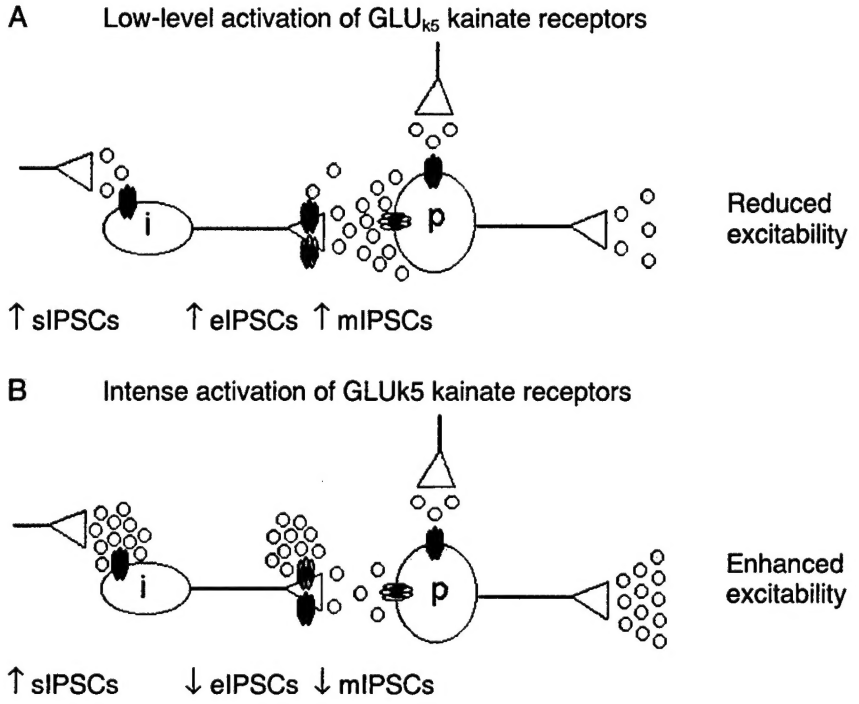

Figure 5